PI Name	Institution	Title	Grant	Years Awarded	Funding Institute
Brahmasandra, Sundaresh	Handylab, Inc.	Nanoliter Device For Hepatitis C Detection	R43 AI051919	2 years	NIAID

HCV is a widely prevalent RNA virus with 200 million individuals worldwide, and 4 million Americans are chronically infected with HCV. Patients with chronic HCV infection are at risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Rapid diagnosis of HCV can lead to early treatment and prevention of transmission. While enzyme immunoassays for the detection of hepatitis C antibody are inexpensive and reliable in the screening of HCV infection, they do not differentiate between recovered and chronic infection, and are of no value in monitoring response to treatment. Qualitative and quantitative reverse transcription-PCR (RT-PCR) assays are available for the diagnosis and monitoring of HCV infected patients but these tests have significant drawbacks of high cost, long analysis times, limited range of linearity, and requirement of skilled labor. We propose to develop a portable, self-contained, microfabricated device for the diagnosis of infectious diseases using real-time polymerase chain reaction (PCR) to detect and quantify the levels of HCV viral load. Such a device could significantly reduce costs and dramatically improve the quality of care of HCV infected patients. Our ultimate goal is to develop a self-contained, inexpensive, microfabricated device that can detect the presence as well as concentration of one or more infectious agents in near-patient setting.

Brousseau, Louis C	Quantum Logic Devices	Single DNA Sequencing With Tunneling Spectroscopy	R43 CA001790	2 years	NCI

This research will create a new technique, capable of direct sequencing of single molecules of RNA, DNA and other oligonucleotides. By combining ultra sensitive quantum dots with scanning probe microscopy, molecular topology is recorded simultaneously with chemical composition at sub-nanometer resolution. This enables determination of the nucleoside sequence as well as the identification of single nucleotide polymorphisms, kinks, dislocations, and other mutations of the strand. The prototype and developed software will allow high throughput and automated sample analysis. This novel tool will be enabling for the realization of genetic-based healthcare (pharmacogenomics). By providing a direct, non-PCR method for genetic sequencing, routine application of this information to genetic predisposition screening, diagnosis, and prescription becomes available for clinical laboratories and hospitals. Expression and mutation studies can also be applied to drug development, as well as for more effective clinical trial candidate selection.

Gao, Jun	Calibrant Biosystems, Inc.	Polymer Nanofluidic Field-Effect Pumping	R43 EB000453	2 years	NIBIB

This proposal describes a research plan for the development of a new technology for nanochannel pumping using a technique pioneered by our research team called Field-Effect Flow Control (FEFC). This technique is based on a modification of standard electronsmotic flow (EOF), with modulation of the flow velocity provided by a secondary gate voltage independent from the lengthwise EOF electric field. The technology allows for direct and independent control of flow within large numbers of interconnected nanochannels, and is expected to impact a broad range of bioanalytical instrumentation. The proposed nanofluidic FEFC pumping technology is a novel approach which will provide nanochannel flow control with specific advantages over other techniques, namely (i) independent operation of multiple pumping elements, (ii) compatibility with standard polymer nanofluidic technologies, (iii) elimination of pump leakage for 100% pump efficiency, (iv) very large scale integration of nanochannel pumping elements for complex nanofluidic systems, and (v) simple fabrication and low cost. The overall goal of this Phase I project is to demonstrate the feasibility of polymer FEFC nanopumping technology, and evaluate its relative merits compared to alternate methods of nanochannel flow control. Practical tools for the analysis of single molecules and other novel bioanalytical instrumentation are anticipated to result from advances in flow control for nanofluidic systems. One example of the potential for this technology is in the sequencing of single strands of DNA by pumping individual molecules through a nanoscale channel, with integrated electrodes interrogating the conductivity of each base (1). Other applications of great interest include nanofluidic devices to mimic biological processes, and to carry out single-molecule chemical reactions.

Josephs, Robert | Arryx, Inc. | Optical Trapping | R43 RR017152 | 2 years | NCRR

Optical trapping with laser tweezers has proven to be a useful device for manipulating objects on a microscopic scale. Many systems of interest require multiple optical traps and several methods have been developed to achieve multiple trap configurations. However all of these methods for increasing the number of traps make the optical system increasingly more complex. Consequently currently available trapping systems can produce at most only a few independent traps. Recently, Grier and Dufresne conceived of a new solution for achieving a multi- trap system. In their method a hologram is used to alter a single laser beam's wavefront. The wavefront is altered so that the downstream laser beam forms a large number of individual laser beams with relative positions and directions of travel fixed by the exact nature of the hologram. The hologram can be calculated from a user specified pattern of desired trap positions. Currently a laboratory prototype has been fabricated. This prototype has demonstrated the ability create the holograms and to trap multiple (up to 200) particles in any chosen p Item. The prototype has not been optimized for use by individuals without expertise in optics. We propose developing a commercial version of the holographic optical tweezers (HOT) along with a sample chamber suitable for introducing, manipulating and collecting specimens.

Lartius, Raj K | Novascan Technologies | Sensitive High Speed AFM Probes For Biology | R43 RR016377 | 2 years | NCRR

The goal of this project is to promote nanoscale biological and biomedical applications that utilize Atomic Force Microscopy. Clearly the future of these technologies is highly reliant on the development of next generation, sensitive, high speed Atomic Force Microscope probes. We are proposing to develop such AFM probes that are optimized for enhanced molecular characterization and biological imaging. These probes will have reduced viscous damping, higher scan rates, and improved sensitivity over current commercially available probes. Additionally these probes will be mass producible, usable in standard commercial AFMs and will be valuable for use in biological application where optical path (i.e. AFM/optical microscopy) and access to the tip area are important (i.e. AFM/electrophysiology).

Niu, Chunming | Nanosys, Inc. | Biomolecule-Gated Nanowire FET Sensors | R43 AI052533 | 2 years | NIAID

The ultimate goal of this project is to develop a hand-held device with a plug-and-play nanosensor platform based on biomolecule-gated nanowire field effect transistor (FET) sensors. The principle of the nanowire FED sensor has been demonstrated by Prof. Charlies Lieber's group at Harvard University. The technology requires no label and combines the specificity of antibody-antigen binding with the exquisitely high sensitivity associated with electrical properties of semiconductor nanowires to achieve near single molecule detection. Our objective in this phase I program is to demonstrate the selectivity and sensitivity of nanowire FET nanosensors for anthrax detection. We will develop chemistry for the immobilization of anti-anthrax antibody on the surface of Si nanowires, establish a process for the fabrication of nanowire FET sensors, optimize electrical signal response of devices upon anthrax maker, protective antigen (PA) binding, and measure the detection limits and dynamic response of the devices at physiologically relevant concentrations.The proposed studies will define fundamental properties of nanowire sensors for selective detection of the biomolecular marker under in-vitro conditions. We believe that these studies will lay the groundwork for developing a very exciting and important program for the NIH. The nanowire nanosensor devices developed in this project will serve as a stepping stone to the creation of minimally-invasive in-vivo sensors for real-time monitoring, and moreover, could be used to develop devices that could simultaneously monitor a large set of protein markers in patients known to be high risk - this could lead to detection at a stage simply not possible today. Lastly, integrated nanosensor arrays could serve as a new tool for discovery and screening in molecular biology with parallelism and sensitivity not possible using any other sensor/detection technology.

Prokop, Ales | Nanodelivery, Inc. | NanobioReactor For Monitoring Small Cell Populations | R43 RR016124 | 2 years | NCRR

We will develop microminiaturized cell-culture environments, i.e., NanoLiter BioReactors (NBR) for growing and maintaining populations of I to 100 cultured mammalian cells in volumes three orders of magnitude smaller than in standard multi-well screening plates. This would reduce the time required for diffusive mixing, thermal equilibrium, and for cells to grow to confluence; simplify accurate cell counting; minimize required volumes of expensive pharmaceuticals or toxins; and allow thousands of culture chambers on a single instrumented chip. Our long-term goal is to develop a new class of miniature, automated cell-based bioanalyzer arrays for monitoring the immediate environment of multiple cell lines and assaying the effects of drug or toxin exposure. This proposal would develop a NBR that detects cellular responses, provides appropriate control signals, and makes closed-loop adjustments of the environment (in Phase II), e.g., by adjusting temperature, pH, ionic concentrations, or by applying another drug or a selective toxin antidote. To characterize in a nonspecific manner the metabolic activity of cells, the biosensor elements of the NBR will include an isothermal picocalorimeter to monitor heat response, and planar pH, dissolved oxygen and redox potential sensors. We will demonstrate short-term and long-term cultivation of several mammalian cell lines and monitor their response to test substances. The proposed technique will enable automated, parallel and multiphasic monitoring of multiple cell lines for drug and toxicology screening. PROPOSED COMMERCIAL APPLICATION: The unique capabilities of this technology would allow analysis of nonspecific responses to an unknown insult. It can lead to massively parallel screening of pharmaceuticals, toxins and other stresses. Once converted into a fully automated system and data analysis and data reduction algorithms are fully developed (in Phase II), this equipment could be readily commercialized, and sold to pharmaceutical companies, research laboratories, and environmental monitoring services.

Reddy, G. R | Molecular Therapeutics, Inc. | Molecular Imaging Of Neoplasia Using Nanoparticles | R43 CA091724 | 1 year | NCI

The long term goal of the present proposal is to develop a nanoparticle that can be targeted to tissues with specific molecular signatures. Inclusion of a contrast agent (Iron Oxide for MRI) within the nanoparticle would therefore facilitate non-invasive imaging of specific biochemical properties within tissues in-vivo. The present proposal is focused on investigating if a nanoparticle based contrast agent can be efficiently targeted to cells with specific molecular characteristics. Two well-characterized molecular targets will be investigated. The first involves targeting to endothelial cells that express alpha-V-beta-3 and alpha-V-beta-5 integrins using the sequence CDCRGDCFC. Expression of these integrins is a hallmark of endothelial cells within angiogenic vasculature (e.g. within tumors). Similarly, we will target tissues (endothelial cells and tumors) that are undergoing tissue remodeling. Since expression of the metalloproteases MMP-2 and MMP-9 is characteristic of cells that need to migrate through the extracellular matrix or basement membrane, inclusion of the CTTHWGFTLC within a nanoparticle will target nanoparticles to tumors and tumor vasculature that typically express these proteases. It is anticipated that the results from the proposed studies will provide the foundation for future development of these novel nano-structure-based strategies for non-invasive in vivo imaging of specific biochemical events.

Sauer, Jon R | Eagle Research And Development, LLC | Silicon-Based, Single Molecule, DNA Analysis Prototype | R43 HG002725 | 2 years | NHGRI

The ultimate goal of this project is a silicon chip with a fabricated, well-instrumented nanopore capable of discriminating between the bases of long strands of DNA. This occurs as they thread through the pore at MHz rates. Discrimination is done through the image charge induced in the gate region of the semiconductor sensors placed to detect the unique, asymmetric charge distributions of single-stranded DNA (ssDNA) at the narrowest part of the nanopore. Such a chip would then be the core device of an inexpensive, ultra-fast sequencing system. This final system would transform diagnostic medicine by enabling exquisitely targeted treatments using sequencing data both from the patient and from the disease-causing pathogen. For example, an AIDS patient violently allergic to some antibiotics could be treated with an anti-viral medicine for the specific mutant AIDS strain affecting him/her. The specific device to be developed in the initial phase contains all the essential elements of the final system - a V-groove pore and local electronics - but is built at the 100 nm scale instead of the 1 nm scale needed to sequence ssDNA. The immediate goal is to obtain bio-compatible, rugged and low-cost nanopores suitable for immersion into a liquid. It will be both a proof-of-principle demonstration and an engineering evaluation of many of the techniques and procedures needed in the final system. This initial device will be fabricated in silicon with state-of-the-art, but well-established, technology including sub-micron feature sizes, V- groove etching, and a vertical architecture. Arrays of 1 - 100 nm size nanopores will be fabricated on a silicon substrate, which also contains local electrodes and electronics to facilitate and enhance the measurements. The fabricated device will be first characterized and tested with larger molecules such as proteins to prepare for designing and constructing the final phase, scaled-down device. Its use will be demonstrated by detecting, identifying and sizing individual DNA molecules and proteins as a necessary step towards developing the sensitivity and resolution required for DNA sequencing.

Seeney, Charles E | Nanobiomagnetics, Inc. | Magnetic Nanoparticles For Advanced Hearing Devices | R43 DC005528 | 1 year | NIDCR

NanoBioMagnetics? objective is to develop and commercialize magnetic nanoparticles as middle ear implants for hearing restoration. Hearing loss currently impairs over 24 million Americans. Emerging implantable electromagnetic hearing device (IHD) technology is proving to be safe and effective. Clinical advancement of IHD technology could be achieved through miniaturization of the magnetic implants. Phase 1 will demonstrate feasibility of preparing hermetic ferromagnetic nanoparticles and attaching them to middle ear ossicles. Two different encapsulation technologies will be evaluated: 1) Thermal Plasma Deposition (TPD): rf-Inductive Plasma will be used to create layered films in which nanoparticles are sandwiched within layers of titanium. 2) Nano-Layer Deposition (LBL): Polyelectrolytes in appropriate media will be used as effective templates for nano-layered films comprised of titanium encapsulated ferrite materials. Overcoating of nanoparticles with collagen Type 1 will be evaluated by implantation onto the middle ear ossicles of guinea pigs. Timed harvesting of tissues, histological staining for iron, and photomicroscopy will document cellular attachment and hermeticity. Phase 2 will scale device production methods, predict human performance using temporal bones and laser interferometry and define clinical applications. Commercialization is available through an Oklahoma IHD company, anticipating FDA approval of their device in August. PROPOSED COMMERCIAL APPLICATION: Hearing loss is the greatest sensory deprivation in any population. Estimates of 25-28 million people in the U.S. have hearing loss. The long term objective of this work is to commercialize lifetime-implantable, magnetically-responsive, nanoparticles to take the place of the larger, more risky and more expensive middle er devices that are being implanted at present, thus making this new rehabilitative technology more affordable and more available to the hearing impaired population.

Szmacinski, Henry K | Microcosm, Inc. | Metallic Nanosensor Matrix With Enhanced Fluorescence | R43 CA097569 | 2 years | NCI

Fluorescence is a dominant technology in medical testing, drug discovery, biotechnology and cellular imaging. There is demand for better fluorescent agents in terms of intensity, spectral range, functionality, photostability and phototoxicity. Recent trends in analytical and material sciences drive devices smaller and faster toward molecular level sensing. At this level, sensitive detection is a major issue.This research promises to provide nanoscale and molecular sensing with simultaneous dramatic increases in fluorescence signal to noise ratio. The sensing technology relies on the interaction of light with metallic nanoparticles such as silver and gold colloids resulting in remarkably high increases in fluorescence intensity of nearby fluorophores of up to 10 Million-fold. The research in development of nanosensors will be focused on fabrication of multifunctional composite nano-sized particles with fluorescent probes. The sensing elements will be constructed using silver and silica nanometer sized colloids combined with fluorescent probes. To demonstrate feasibility of the nanosensors, probes for pH and Ca2+ measurements will be used.Several modern optical techniques will be used to evaluate effects of fluorophore-metal surface interactions including laser scanning, two-photon excitation, and lifetime imaging microscopy. Software will be developed for rapid acquisition and image analysis of 2D and 3D nanosensors. The result of Phase I will be a basis of knowledge sufficient to permit prototype sensor fabrication and continued development in Phase II. Probe-metal colloid composites will significantly improve detection and analysis of biologically relevant analytes in samples including whole blood, saliva, and other body fluids. These novel sensors with improved intensities and photostability can be used with or without imaging optics. Due to the predicted large enhancement in fluorescence intensity, we envision the future development of field deployable biosensors to rapidly detect environmental infectious agents with sensitivity and specificity approaching if not surpassing laboratory procedures.

Turner, Stephen W | Nanofluidics, Inc. | Nanofluidics-Based DNA Screening Device | R43 HG002548 | 1 year | NHGRI

A nanofluidic DNA screening device for cancer-related diagnosis and research will be developed. Point mutations and single nucleotide polymorphisms will be detected optically using fluorescent hybridization probes on a fully stretched strand of chromosomal DNA as it passes through a nanoscale tube. Array-based methods for detecting single-base differences have been demonstrated, but the fluidics method proposed here has several advantages, including immunity to coincidental sequence repeats, superior detection of rare mutations in mixed samples, and the retention of correlations between different loci on the same chromosome. Fluidics-based methods for screening genetic content have been studied before (Austin 1999), but have been limited by the methods of fluidics fabrication employed. For this device, a sacrificial layer technique for fabricating fluidics has been developed which allows precise control of complex fluid circuits with nanoscale dimensions (Turner 1998). A tube smaller than the persistence length of double-stranded DNA will prevent the molecule from folding back on itself while passing through the tube, thus eliminating the primary source of error in previous attempts to implement this method.

Van Der Weide, Daniel | Prairie Technologies, Inc. | Noncontact Membrane Protein Probe | R41 MH065808 | 2 years | NIMH

Reading the conformational state of membrane proteins is central to understanding their role in cell signaling, to using them as sensor elements and for screening candidate drug compounds that are targeted toward this important class of biomolecules. While the patch clamp technique for doing this is ubiquitous, it does have significant limitations: two electrodes are required to measure current flow, the GW input impedance combines with unavoidable stray capacitance to limit the amplifier's bandwidth (typically to -10 kHz), and the GW seal required at the cell membrane precludes scanning to image both distribution and dynamics of membrane proteins. To address these limitations, we will develop near-field probes to confine high-frequency excitation to sub-wavelength proportions, enabling interaction with single membrane proteins. Since there are inherent dielectric contrast mechanisms available in protein-lipid systems, these can be used at high frequencies to provide a new method of protein readout. The overall goal of this work is to build scanning protein probes that can image distribution and dynamics of ion channel activity simultaneously.