Optical Instrumentation Systems

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OPTICAL IMUNOASSAY SYSTEM USING DISPOSABLE PLANAR WAVEGUIDES

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We have developed a fluorescent-based system for detecting low-level anayltes in point -of-care and critical care settings. The goals of the design were: 1) high sensitivity, comparable to present clinical chemistry analyzers, 2) rapid response times (5 minutes) with little operator attention, and 3) disposable, one-shot sample modules. We achieved these goals by using a molded plastic waveguide as the solid substrate for immobilizing the capture antibodies, a diode laser as excitation source, and a CCD detector for collecting the fluorescent signal. In sample solutions of BSA, hCG and ovalbumen, the system has demonstrated a sensitivity of approximately 10 pM. This work was supported in part by HCP Diagnostics, L.P.

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PRELIMINARY SENSITIVITY STUDY OF REAL-TIME PROTEIN C BIOSENSOR

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A real-time, portable, and highly sensitive biosensor for Protein C (PC) is being developed. Briefly, an optical fiber is enclosed in a 300 µl sample chamber. Monoclonal antibodies to PC are immobilized on the fiber surface. PC in a sample is allowed to specifically interact with the fiber. Bound PC is probed with a fluorophore tagged secondary antibody to PC. Excitation light is generated through the fiber, and PC concentration is directly correlated with the intensity of fluorescence. The current paper focuses upon increasing biosensor sensitivity by examining optimal probe antibody concentrations. Biosensor performance is observed when varying probe antibody concentrations are used with PC samples mixed with a nonspecific competitor, human serum albumin (HSA). HSA is used to model natural conditions. Optimization of this system may lead to the detection of PC deficiency in a clinical setting.

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AN ENHANCED MICRODEGREE POLARIMETER FOR GLUCOMETRY IN BIOTECHNOLOGY APPLICATIONS

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A Gilham-based polarimeter uses crossed polarizers and E vector modulation to measure the amount of rotation of polarized light caused by an optically active solution in a known path length. In the past, such systems have required the use of an expensive Faraday rotator and a high-voltage photomultiplier tube (PMT) to obtain resolutions down to the microdegree range. We have developed a modified low-cost closed-loop Gilham polarimeter with microdegree resolution using a coil wound around the solution-under-test instead of a Faraday rotator, and a silicon photo-diode in place of a PMT. We increased sensitivity and decresed instrument size in the current version by changing our light source to a shorter wavelength solid-state laser. Preliminary results have shown a resolution of better than 15 microdegrees per 10 millivolts output. This translates to a glucose concentration sensitivity of 20 millivolts per milligram percent.

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THREE-DIMENSIONAL IMAGING OF THE SEPARATION SURFACE BETWEEN CONVERGING FLOWS AT AN ARTIFICIAL VENULAR BIFURCATION

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We describe a new methodology which uses a combination of confocal microscopy and microfabrication to measure the shape and position of the separation interface between two converging flows at an artificial venular bifurcation. In this study, a T-type bifurcation is constructed by fabricating glass microchannels with semicircular cross sections (107 micron diameter, 50 micron depth) using photolithographic methods. A simplified model of blood flow is examined for two converging Newtonian fluids. The separation surface is visualized using a laser scanning confocal microscope and three-dimensional image reconstruction. Optical aberrations inherent in the apparatus must be considered in order to produce a quantitatively accurate reconstruction. Experimental results are validated using three-dimensional finite-element simulations (FIDAP 7.06) on a computational domain which replicates the microchannel geometry.

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A BINDING ASSAY USING AEQUORIN AS A BIOLUMINESCENT LABEL TO MEASURE FEMTOMOLAR LEVELS OF INTRACELLULAR BIOMOLECULES.

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Characterizing the activities of single cells requires the identification and quantification of intracellular biochemical changes of biomolecules, such as proteins, vitamins, drugs, and toxins. Conventional biochemical analyses measure an average composition of thousands of cells, yet the distribution of substances varies within a given population. Therefore, there is a need for analytical procedures that are capable of measuring analytes in single cells. It has been demonstrated that the Ca^{2+}-triggered luminescence reaction of biotinylated recombinant aequorin can be inhibited by the presence of avidin. This inhibition in bioluminescence intensity has been used to detect biotin concentrations as low as 10^-14 M in a sample size of 400 µL. The assay has been performed with a sample of lysed fibroblasts in a volume of approximately 350 pL. Further scale down of the assay to single cells will be discussed.

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EXTERNAL CAVITY LASER DIODES FOR USE IN MEDICAL RAMAN APPLICATIONS

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Raman scattering is a powerful technique which can be used for many medical applications, including blood substance monitoring for anesthetics. The source required for Raman scattering must have high power, good power stability, and a spectral output of a few GHz. Single-stripe laser diodes have become available recently which are relatively inexpensive and emit powers greater than 1 W. However, the spectral output is too broad for use in Raman scattering. We show several techniques which can be used to produce narrow linewidth, high power sources, by placing these laser diodes in an external cavity. Experimental results are shown.