

To these and other ends, the present invention includes a method of detecting and quantitating an analyte, including the method for detecting and quantitating a small quantity of an analyte, the method comprising the following steps: providing a laser light source for emitting a beam of laser light; providing first and second bodies, at least one of the first and second bodies being adapted to be manipulated by the beam of laser light; adhering the analyte to the first body; adhering to the second body a reagent that is reactive with the analyte; bringing the first and second bodies into sufficient proximity to cause a reaction between the analyte and the reagent; separating the first and second bodies by use of the beam of laser light; determining a force necessary to carry out the separating step; and determining a quantity of the analyte from the force determined in the determining stop.

The present invention also includes an apparatus for detecting and quantitating a small quantity of an analyte, the apparatus comprising the following elements: a laser light source for emitting a beam of laser light; first and second bodies, at least one of the first and second bodies being disposed in the path of the beam of laser light and being adapted to be manipulated by the beam of laser light; means for causing the analyte to adhere to the first body; a reagent that is reactive with the analyte, the reagent being adhered to the second body; means for separating the first and second bodies, after the analyte and the reagent have reacted, by use of the beam of laser light; means for measuring the force that the means for separating must exert to separate the first and second bodies; and means for calculating, on the basis of the force, a quantity of the analyte.

In their experiments, the Applicants coated 4.5 micron latex spheres with antigen. Using the latex spheres as handles, it is possible to pull on the antigen molecules with optical tweezers. The average minimum laser power required to pull the sphere into the trap and break the bonds between the antigen and either non-specific protein or specific antibodies coated on the glass coverslip was measured. The resulting experimental data indicated that more laser power, hence more force, was required to break the specific antigen/antibody bond versus the non-specific binding cases. This was observed over several orders of antigen concentration, even at the level where we were measuring a single antigen/antibody bond. Similarly, this same process could be followed for the interaction of complementary DNA probes on the latex particle and the glass slide.

A sensitive assay has been developed using this technique by initiating a competitive binding situation between the antigen or DNA sequence on the bead and a similar material in the added sample. These components compete for the binding component attached to the glass slide.

Those skilled in the art who have reviewed this specification will readily appreciate that a very sensitive assay for proteins, organic molecules, receptors, and nucleic acid sequences is possible using this technology. It will also be apparent to those skilled in the art that this assay will detect particulate antigens such as bacteria, viruses and cell components. Those skilled in the art will further appreciate that this assay is able to quantitate analytes within an order of magnitude, with more sensitive quantitation achievable upon optimization of the assay for a particular analyte.

This technology should find major application for detection of infectious diseases, through the detection of disease specific antigens, antibodies, or nucleic acid sequences. It should also find application in detection or any other component presently detectable through PCR, DNA probe tech-

nology or immunoassay but at the level of only a few molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

The preferred embodiment of the invention will now be described in greater detail with reference to the drawings, in which:

FIG. 1 shows a schematic diagram of an apparatus according to the present invention; and

FIG. 2 shows experimental results of Titer Curve for BSA (67,000 MW) coated beads at decreasing concentrations of BSA with constant antibody (Ab) concentration on the glass coverslip in accordance with the present invention.

FIG. 3 shows this data recalculated to show the average number of antigen molecules per bead.

FIG. 4 shows data for a separate binding component using mixed silanes for coupling the antibody to the glass coverslip.

FIG. 5 is a graph showing competitive binding of soluble antigen vs. immobilized antibody/antigen bead at two concentrations of antigen immobilized on the beads.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

An apparatus according to the present invention is shown in FIG. 1. This figure shows a schematic diagram and is not drawn to scale.

As seen in FIG. 1, the apparatus uses a focused laser beam from a laser light source, such as laser 3 and lens 5 to trap bead 7. The laser should operate within the visible or infrared spectrum. The preferred visible light frequency should be between 375–800 Angstroms. The infrared frequency should be between 800–2500 Angstroms. The size of the bead may be between about 0.5 μ about 100 μ in diameter. In addition, the bead should comprise a material that does not adsorb the wavelength of the laser beam to prevent the bead from heating during operation of the apparatus. The bead can be moved with the laser under the control of moving means 9. The force needed to move the bead is measured by force measuring means 11. The measured force is outputted to calculating means 13, which uses the measured force to determine the presence and quantity of an analyte, as will be explained in greater detail below.

The bead and second body 15 such as a glass coverslip are exposed to a solution containing the analyte that is placed in well 17. To prevent undesirable heating of the solution, the solution should not absorb the wavelength of the laser beam. To prevent the solution from undesirable heating during operation of the apparatus, the solution should not adsorb the wavelength of the laser beam. The glass cover slip has adhered thereto molecules of reagent (not shown) which react with the analyte. Molecules of the analyte (not shown) are adhered to the bead, preferably with second reagent (not shown). By allowing the analyte to react with the reagent and then measuring the force needed to separate the bead from the glass cover slip, the quantity of the analyte can be determined.

The invention will now be described in further detail with reference to the following illustrative examples.