

OPTICAL TRAP FOR DETECTION AND QUANTITATION OF SUBZEPTOMOLAR QUANTITIES OF ANALYTES

FIELD OF THE INVENTION

This invention is directed to a method and device for the detection of sub-zeptomolar quantities of analytes, including nucleic acids, antigens (either soluble or as components of bacteria or viruses), antibodies, receptor molecules, lectins and other binding pairs (protein, carbohydrate, organic molecules, etc.), by optical trapping.

BACKGROUND OF THE INVENTION

Tightly focused beams of laser light can be used to trap and remotely manipulate polarizable objects. Originally proposed for the trapping of atoms, such devices are also capable of trapping macroscopic, polarizable objects such as latex and glass spheres in the micron size range as well as biological material such as viruses, bacteria, yeast and protozoa, ranging in size from 20 nm to 100 microns. The non-invasive trapping and manipulation of such object have led to the name "optical tweezers" for such devices. The basic principle behind optical tweezers is the gradient force of light which manifests itself when a transparent material with a refractive index greater than the surrounding medium is placed in a light intensity gradient. As light passes through the polarizable object, it induces fluctuating dipoles in the material. These dipoles interact with the electromagnetic field gradient, resulting in a force directed towards the brighter region of the light. Hence the object is pulled into the focus of the laser beam which is the local maximum of the light rigid. Typically, the focus of the laser beam is kept fixed (on the order of the wavelength) so the strength of the trapping force is proportional to the light intensity.

One of the more significant applications of optical tweezers is as a tensiometer. By pulling with the optical tweezers, one can measure the forces associated with certain biomolecular interactions, such as the torsional compliance of bacterial flagella, or the force of single motor molecules like myosin and kinesin. In the later case, the kinesin molecules were attached to micron-sized silica beads with sufficiently sparse surface coverage such that, on average, only one molecule was in contact with a microtubule. Using the silica bead as a handle to pull with the optical tweezers, the force exerted by a single kinesin molecule was observed.

Examples of the devices used in this art and of other relevant work are shown in the following references:

- U.S. Pat. No. 4,893,886 to Ashkin et al, Jan. 16, 1990;
- U.S. Pat. No. 5,100,627 to Buican et al, Mar.31, 1992;
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- Nishizawa, T., Miyata, H., Yoshikawa, H., Ishiwata, S., "Mechanical Properties of Single Protein Motor of Muscle Studied by Optical Tweezers," *Biophysical Journal*, Vol. 68, No. 4, 1995, p. 75s;
- Kuo, S.C., Ramanathan, K., Sorg, B., "Single Kinesin Molecules Stressed with Optical Tweezers," *Biophysical Journal*, Vol. 68, No. 4, 1995, p. 74s;
- Amos, G., Gill, P., "Optical Tweezers," *Measurement Science & Technology*, Vol. 6, No. 2, 1995, p. 248;

- Felgner, H., Mueller, O., Schliwa, M., "Calibration of light forces in optical tweezers," *Applied Optics*, Vol. 34, No. 6, 1995, p. 977;
 - Liang, H., Wright, W. H., Rieder, C. L., Salmon, E. D., "Directed Movement of Chromosome Arms and Fragments in Mitotic Newt Lung Cells Using Optical Scissors and Optical Tweezers," *Experimental Cell Research*, Vol. 213, No. 1, 1994, p. 308.
 - Wright, W. H., Sonek, G. J., Barns, M. W., "Parametric study of the forces on microspheres held by optical tweezers," *Applied Optics*, Vol. 33, No. 9, 1994, page 1735;
 - Wright, W. H., Sonek, G. J., Berns, M. W., "Radiation trapping forces on microspheres with optical tweezers." *Applied Physics Letters*, Vol. 63, No. 6, 1993, p. 715;
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 - "Atomic fountains; laser tweezers; optical molasses," *IEEE Micro.*, Vol 12, No. 4, 1993, pp. 88-89;
 - Block, S. M., "15. Optical Tweezes: A New Tool for Biophysics," *Modern Cell Biology*, Vol. 9, 1990, p. 375;
 - Dai, J., Sheetz, M.P., "Mechanical properties of neuronal growth cone membranes studied by tether formation with laser optical tweezers." *Biophysical Journal*, Vol. 68, No. 3, 1995, p. 988;
 - Afzal, R. S., Treacy, E. B., "Optical tweezers using a diode laser," *Review of Scientific Instruments*, Vol. 63, No. 4, 1993, pp. 2157-2163;
 - Ashkin, A., "Trapping of atoms by resonance radiation pressure," *Physical Review Letters*, Vol. 40, 1978, pp. 729-32;
 - Ashkin, A., Dziedzic, J. M., Bjorkholm, J. E., Chu, S., "Observation of a single-beam gradient force optical trap for dielectric particles," *Optics Letters*, Vol. 11, 1986, pp. 288-90;
 - Ashkin, A., Dziedzic, J. M., Yamane, T., "Optical trapping and manipulation of single cells using infrared laser beams," *Nature*, Vol. 330, 1987, pp. 769-71;
 - Ashkin, A., Dziedzic, J. M., "Optical trapping and manipulation of viruses and bacteria," *Science*, Vol. 235, 1987, pp. 1517-20;
 - Block., S. M., Goldstein, L. S. B., Schnapp, B. J., "Bead movement by single kinesin molecules studied with optical tweezers," *Nature*, Vol. 348, 1990, pp. 348-52.
- The disclosures of these references are hereby incorporated by reference in their entirety into this specification.

SUMMARY OF THE INVENTION

An object of the invention is to provide an apparatus and method for detecting and quantitating minute quantities of analytes such as nucleic acids, antigens, and antibodies, receptors and lectins.

A further object of the invention is to provide an apparatus and method for detecting and quantitating such minute quantities when they are in very low concentration.