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Analytical particle measurements in an optical microflume

Joseph D. Taylor, Alex Terray, Sean J. Hart*

Naval Research Laboratory, Chemistry Division, Bio/Analytical Chemistry Section, Code 6112 4555 Overlook Ave. S.W., Washington, DC 20375, USA

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ABSTRACT

In this work, microscopic particles in a fluid flow are manipulated using forces generated by a high power laser beam. The resulting manipulations on the particles are imaged using a microscope lens connected to a CCD camera. Differential forces on particles of varying physical and chemical composition have been measured. The goal is to measure the optical forces on a diverse range of particles and catalog the associated chemical and physical differences to understand which properties and mechanisms result in the largest force differentials. Using these measurements our aim is to better understand differences between similar microspheres in terms of size, morphology, or chemical composition. Particles of the same size, but different composition show large variations in optical pressure forces and are easily discernable in the present analytical system. In addition, we have demonstrated the ability to differentiate a 70 nm size difference between two NIST precision size standard polystyrene microspheres, corresponding to a 2.0 pN difference in optical force. Lastly, the instrument was used to measure differences between biological samples of similar size, demonstrating the ability to make precise analytical measurements on microorganism samples.

1. Introduction

The interaction between photons and microscopic particles induces optical pressure by imparting a fraction of their momentum when they scatter at the surface or refract through a particle. This effect is considerable when using a collimated light source such as a laser, given the remarkably high number of photons available. The more well-known optical micromanipulation technique known as optical tweezers utilizes a single highly focused laser for tasks such as trapping [1,2] and sorting cells [3,4], or a combination of laser beams working in unison for the development of unique non-intrusive tools such as microactuators [5,6]. The beams can be tightly focused into the solution that is transporting the microscopic particles through a microchip or flow cell. The translucent particles experience a net restoring force that traps them at the laser's focal point, a result of photon momentum transfer from the sharply converging light leaving a microscope objective. By either altering the aqueous environment or repositioning the laser beam, micron-sized particles can be maneuvered and manipulated in real-time in a highly controlled manner.

In a mildly focused laser beam, micron-sized particles experience a radiation pressure force in the direction of the laser propagation and towards regions of high intensity along the beam waist. Kim et al. recently devised a straightforward cross-type optical particle separator with broad applicability toward sorting and separating biological cells at constant velocity. A mixture of 5 and 10 μm particles flowing through a microchannel would encounter a mildly focused laser beam propagating perpendicular to the flow and would be deflected from their original path depending on their size [7,8]. Novel optical force switches also incorporating less divergent beams with longer depths-of-focus have been developed to sort mammalian cells in microfluidic chips by essentially pushing them laterally [9]. Conventional optical chromatography (OC) relies on this force when balanced against an opposing fluid flow [10]. In this case, the diverging portion of the laser beam (i.e. some distance from the focal point) is carefully aligned within and along a capillary separation channel in such a way as to direct the beam toward the approaching fluid carrying the incoming microparticles. When the optical pressure exerted on the particles equals the fluid drag force, particles become trapped within the beam in a stable manner and in unique positions along the beam axis. Particle positioning depends on size [11] and shape [12], but also arises due to differences in refractive index [13,14]. These larger size and/or greater refractive index particles encounter a pronounced optical pressure resulting in their being pushed further down the length of the beam and requiring less photon density relative to smaller or less refractive particles. Using this approach allows the unique separation and retention of various particles on the basis

Abbreviations: OC, optical chromatography; PS, polystyrene; PMMA, poly(methylmethacrylate); Si, silica; MF, melamine formaldehyde resin; *Ba*, *Bacillus anthracis* (Sterne strain); *Bt*, *Bacillus thuringiensis*; Geobacter, *Geobacter sulfurreducens* (strain DL-1).

* Corresponding author. Tel.: +1 202 404 3361; fax: +1 202 404 8119.
E-mail address: sean.hart@nrl.navy.mil (S.J. Hart).

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of their intrinsic and/or extrinsic properties, from polymeric beads and silica-based spheres to biological particles such as pollen and pathogenic bacteria [15,16].

Accordingly, recent research concerning optical manipulation has attempted to understand the influence of composition on the optical pressure of particles under study, rather than merely a size-based dependence. Our group has recently demonstrated that microbiological separations can be achieved within a microfluidic device incorporating optical forces as a means to interrogate minute dissimilarities in a heterogeneous sample containing co-injected microbes [17]. In this particular case, two closely related genetic relatives, *Bacillus anthracis* and *Bacillus thuringiensis*, displayed large differences in their retention distances. This report and others [18] have provided keen insight into the separation of biological particles in a laminar flow system based on intrinsic physical and biological characteristics. Understanding these optofluidic force differentials may result in new avenues to separate biological species using a sorting methodology that exerts no physical contact on the material. Other reports have examined the influence of such intrinsic properties as refractive index by studying the laser-trapping properties in a controlled manner using synthetic microspheres of known refractive index [19].

Previously, optical chromatography research had demonstrated partial optical separation of a fraction of injected particles based upon size [11]. Our laboratory extended optical chromatography for size-independent separation of polymeric particles based on variations in refractive index [13]. Herein we demonstrate a novel analytical technique based upon optical chromatography for obtaining measurements of the optical force required to trap a particle at a set position within a microfluidic separation channel. Holding the laser power constant, a single particle may be retained at a pre-defined position within the channel by adjusting the flow rate, thus creating an optical microflume. Each particular particle becomes trapped with a unique and highly reproducible flow rate, which is used to calculate the fluidic drag force. Since the two opposing forces are balanced when the particle is stationary, the drag force equals the optical force acting on the particle. This approach has demonstrated the ability to easily differentiate between various polymeric and inorganic microbeads of the same size, as well as the ability to discern minute differences in size of particles of the same material (70 nm). To further demonstrate the instrument's capability, genetically similar biological samples of *B. anthracis* and *B. thuringiensis*, as well as wild-type *Geobacter sulfurreducens* bacterial cells have been analyzed.

2. Experimental

2.1. Materials

The primary materials studied consisted of two NIST traceable polystyrene (PS) precision size standard spheres (1.80 ± 0.04, 1.90 ± 0.03 μm) as well as 1.97 μm PS, 1.98 μm poly(methylmethacrylate) (PMMA) microbeads, and 2.00 μm silica (Si) beads, which were all obtained from Polysciences (Warrington, PA, USA). The 2.03 μm melamine formaldehyde resin (MF) particles were from Corpuscular, Inc. (Cold Spring, NY, USA). Si, PMMA, PS, and MF have refractive indices of 1.43, 1.49, 1.59 and 1.68, respectively. Phosphate buffered saline 1× (PBS) was from Invitrogen (Carlsbad, CA, USA) and was used as the carrier liquid for the biological samples. *B. anthracis* avirulent strain Sterne lacking the pXO2 plasmid was previously obtained from the Colorado Serum Company, Denver, Co. and *B. thuringiensis* serovar, *kurstaki* strain 4D7 was obtained from Bacillus Genetic Stock Center at The Ohio State University (Columbus, OH, USA). Sporulation and spore purification are described in detail in a previous report [17].

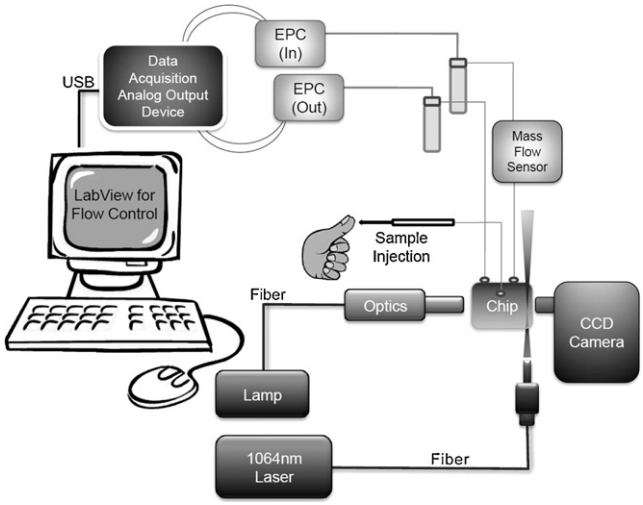

Fig. 1. System schematic showing electronic pressure controllers that control the inlet and outlet pressure in 20 mL vials for precise fluidic pumping. Flow is monitored through a CMOS mass flow meter anterior to the microchip. A near infrared laser is mildly focused using a 0.5 in. plano-convex 100 mm focal length lens into the analysis channel (55 μm) where the width of the beam expands to nearly fill the channel.

Wild-type *G. sulfurreducens*, strain DL-1 (Geobacter) bacterial cells produced with pili were provided by Dr. Bradley Ringeisen at the NRL (Code 6113).

2.2. Instrumentation

The primary components of the optical chromatography system illustrated in Fig. 1 consisted of a continuous wave (CW) 1064 nm ytterbium fiber laser (IPG Photonics, Oxford, MA, USA), an electronically controlled pneumatic pumping system governed by LabView (National Instruments, Austin, TX, USA), a liquid mass flow SLG1430 sensor (Sensirion, Staefa, Switzerland) coupled with nanofluidic connections and tubing from Upchurch Scientific (Oak Harbor, WA, USA), and a custom made microfluidic network with nanoport connections to an inlet, outlet and injection line [20]. The microchip was mounted onto a plexiglass plate fitted onto a 4-axis positioner, while the camera, PL-800 fiber optic illuminator (Edmund Optics, Barrington, NJ, USA), and laser fiber were each fitted to an *x*–*y*–*z* translation platform, all from Thorlabs (Newton, NJ, USA).

The optically transparent microfluidic flow cell had a liquid volume of approximately 500 nL. The chip was bonded together in a sandwich fashion using three fused silica plates with laser-etched channels and inlets as described in a previous report [21]. Imaging was performed using a Retiga 1300 12-bit cooled color CCD (QImaging, Surrey, BC, Canada) coupled with a 20× microscope objective from Olympus (Center Valley, PA, USA) with a focused white light source behind the separation channel. Data were collected using ImagePro Plus 6.0 (Media Cybernetics, Bethesda, MD, USA) to measure the position of the optically trapped microparticles in the liquid flow. The diameter of the separation channel was 55 μm, while the length was 500 μm. The laser light was focused through a 0.5 in. diameter plano-convex 100 mm focal length lens and aligned such that the width of the beam filled the separation channel.

MilliQ water and/or PBS were pumped through the flow cell and precisely controlled using Pneutronics miniature electronic pressure controllers (EPC) from Parker-Hannifin (Cleveland, OH, USA) capable of delivering steady, pulse-free liquid flow using nitrogen gas. Each EPC controlling the gas directed over the "inlet" and "outlet" liquid reservoirs was regulated by a 4P4C breakout board with an R/A connector from Winford Engineering (Bay City, MI, USA),