

## Cascade optical chromatography for sample fractionation

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Optical chromatography involves the elegant combination of opposing optical and fluid drag forces on colloidal samples within microfluidic environments to both measure analytical differences and fractionate injected samples. Particles that encounter the focused laser beam are trapped axially along the beam and are pushed upstream from the laser focal point to rest at a point where the optical and fluid forces on the particle balance. In our recent devices particles are pushed into a region of lower microfluidic flow, where they can be retained and fractionated. Because optical and fluid forces on a particle are sensitive to differences in the physical and chemical properties of a sample, separations are possible. An optical chromatography beam focused to completely fill a fluid channel is operated as an optically tunable filter for the separation of inorganic, polymeric, and biological particle samples. We demonstrate this technique coupled with an advanced microfluidic platform and show how it can be used as an effective method to fractionate particles from an injected multicomponent sample. Our advanced three-stage microfluidic design accommodates three lasers simultaneously to effectively create a sequential cascade optical chromatographic separation system. © 2009 American Institute of Physics. [doi:[10.1063/1.3262415](https://doi.org/10.1063/1.3262415)]

### I. INTRODUCTION

Laser radiation focused at microscopic particles in a fluid can generate significant forces on the suspended particles through the momentum exchange that occurs when photons from the laser refract through and reflect off of the particles. By manipulating these forces in novel ways, microscopic particles can be trapped, manipulated, and sorted.<sup>1–5</sup> The most familiar use of this phenomenon is termed optical tweezers or optical trapping and can employ one or more laser radiation sources. Of the forces resulting from highly convergent Gaussian laser radiation, the dominant restoring force is that which retains particles at the focal point of the converging rays. By translating the particle medium or the laser beam, individual particle translation and manipulation can be achieved. This method has been used successfully to separate microscopic particles based on their visual appearance, such as size, shape, and fluorescence. More recent work has utilized arrays of optical traps to separate particles based on their different optical mobilities.<sup>6–8</sup> Other techniques involving novel methods of manipulating the incident beam have increased the complexity and success of unique separations.<sup>9,10</sup>

Optical chromatography<sup>11–13</sup> relies on a mildly convergent Gaussian laser beam to draw particles toward and propel them along its axis of propagation. The laser is aligned to directly oppose a fluid flow; when the fluid drag and optical pressure forces on incident particles are balanced, they are retained in the system. Larger size and greater refractive index particles each experience greater optical pressure and are retained further from the focal point than smaller or lower refractive index particles. This results in unique retention distances from the focal point for particles of

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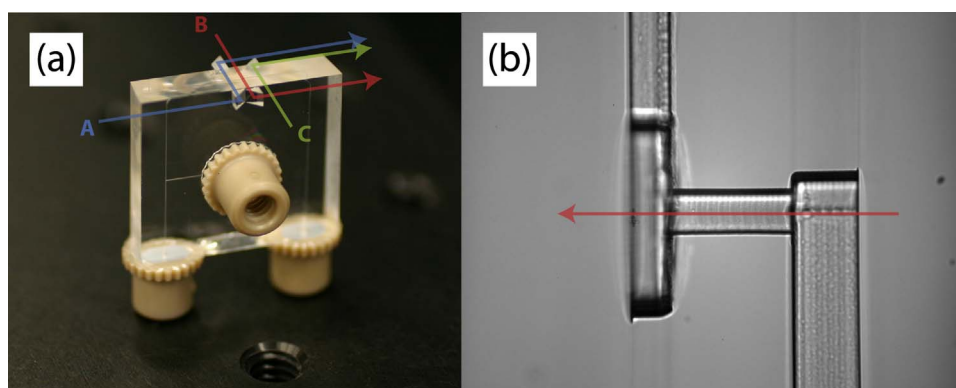


FIG. 1. Cascade optical chromatography flow cell. (a) Picture of the microfluidic device showing the three separation regions and the prisms. (b) Microscopic image of one of the three separation regions viewed from the top down (capillary diameter is 50  $\mu\text{m}$ ).

different sizes and compositions (refractive index). Using this technique it has been demonstrated that particles and biological microorganisms can be separated by size,<sup>11,14</sup> refractive index,<sup>15</sup> shape and morphology,<sup>16</sup> and fluid drag characteristics.<sup>16</sup>

In this paper we demonstrate a new separation method based on optical chromatography for the complete fractionation of two three-component colloidal mixtures. In recent optical chromatography research,<sup>11–15</sup> a lightly focused laser has been used to collect both analytical data about an interrogated particle and if desired, fractionate, concentrate, and collect the sample.<sup>17,18</sup> The goal with this new implementation of optical chromatography is to perform complete sample fractionations. The ability to fractionate and/or purify a sample can be advantageous for application with existing technologies that require sample cleanup. Earlier work used a single laser to retain and purify *B. anthracis* (B.a.) spores from chemical interferents, such as humic acid before real-time polymerase chain reaction (RT-PCR) DNA analysis was run on an externally collected sample.<sup>18</sup>

## II. EXPERIMENTAL

The optofluidic fractionation device discussed here consists of a fluidic apparatus encompassing all liquid handling, including pumping and injections, coupled with an optical system. The combined result makes it possible to have three independently controllable continuous wave (CW) 1064 nm ytterbium fiber lasers (IPG Photonics, Oxford, MA, USA) simultaneously focused into three sequential channels in a fused silica microfluidic device. Each laser is focused by a 0.5 in. diameter planoconvex, 100 mm focal length lens. The microfluidic network is mounted on a five axis positioner (New Focus, San Jose, CA, USA) and the entire aligned optic and fluidic system is observed using a 20 $\times$  objective and lens tube system (Infinity Photo-Optical, Boulder, CO, USA) connected to a charge-coupled device (CCD) camera (Olympus America, Inc., Center Valley, PA, USA) mounted independent of the optic and fluidic components.

The microfluidic flow cell, similar to previous work,<sup>18,19</sup> shown in Fig. 1, consists of three plates of fused silica. Rather than etching all three plates, only the 250  $\mu\text{m}$  thick center plate has been wet etched in hydrofluoric acid (HF) to yield a structure of etched trenches, 85  $\mu\text{m}$  wide and 40–60  $\mu\text{m}$  deep, on both sides of the plate with three 50  $\mu\text{m}$  diameter capillary through holes connecting the trenches on either side of the plate (Translume Inc., Ann Arbor, MI, USA). This layer is bonded between two 2 mm thick outer plates at room temperature,<sup>20</sup> into which have been drilled 350  $\mu\text{m}$  diameter holes that align with the inlet, outlet, and injection channels on the central layer. The bonded device is shaped and polished using a diamond lapping machine (Crystalite Corp., Westerville, OH, USA). Fluid connectors (Nanoport, Upchurch Scientific, Inc., Oak Harbor, WA, USA) were epoxied to the finished chip to allow for fluid inlet, outlet, and injection tubing. The fabricated microfluidic chip has a final design incorporating three optical chromatog-

raphy regions, each separated by 0.5 mm. To align and focus three laser beams into this chip design, 0.5 mm prisms were glued (using Norland optical lens cement) to the surface of the microfluidic chip (Fig. 1), in an orientation that allowed for each laser to enter along a perpendicular axis to the previous. The final system utilizes all six major axis directions and includes the illumination source, a 20 $\times$  objective with CCD camera for observation, lasers A–C, and an exit for all three lasers along the final axis.

The fluid control system consists of a pneumatically controlled reservoir involving very precise pressure control over a 20 mL volume of pure water. The liquid volume was connected to tubing resulting in pulseless, stable, and reproducible fluid flow. Computer control via an electronic pressure controller (OEM-EP, Parker Hannifin, Hollis, NH, USA) allowed for rapid and interactive manipulation of the pressure and thus flow rate. The complete system involved connecting the inlet and outlet tubing each to a separate reservoir. The dual reservoir system completely isolated the flow system increasing the stability and added the ability to control flow direction. Flow direction and flow rate were precisely measured to a resolution of 0.5 nL/min using a calibrated commercial liquid mass flow meter (Sensirion Inc., Westlake Village, CA, USA).

Sample injections were made using a syringe pump (NE-1000, New Era Pump Systems Inc., Farmingdale, NY, USA) fitted with a 10  $\mu$ L syringe (Hamilton, Reno, NV, USA). Each of the two mixture samples consisted of three components with the first containing 10  $\mu$ m polystyrene (PS) spheres, 2  $\mu$ m poly(methyl methacrylate) (PMMA) spheres, and 1.5  $\mu$ m silica (Si) spheres (Polysciences, Inc., Warrington, PA, USA) and the second mixture containing 12–13  $\mu$ m paper mulberry pollen (Polysciences, Inc., Warrington, PA, USA), *B.a.* (avirulent strain Sterne lacking the pXO2 plasmid was previously obtained from the Colorado Serum Co., Denver, CO, USA), and *E. coli* cells (ATCC 11229).

To better understand the process taking place in each fractionation region we employed a method involving the numerical simulation of particles trapping in our system. Our simulation was conducted using the commercially available software package FLUENT (Ansys, Inc., Canonsburg, PA, USA) and has been described elsewhere.<sup>19</sup> Figure 2 illustrates the typical interaction of forces found in our separations. In the figure, a particle mixture is sorted where the incoming fluid flow turns and enters a 50  $\mu$ m channel filled with a concentrically aligned laser directed in the opposite direction to the flow. Inhomogeneous colloidal mixtures contain particles with specific physical (size and shape) and chemical (refractive index) differences that result in substantially different optic and fluidic forces. These can be manipulated by changing the laser power and flow rate, and as a result, some optimization was required to achieve the best possible fractionation.

For each experiment, we utilized a procedure to optimize and maximize fractionation efficiency. The calibration process involved injecting pure samples of the components that made up the mixed sample and adjusting the laser power in each separation region to maximize the fractionation of each component, while minimizing the contamination from any of the other components. In each case the component exhibiting the lowest optical mobility was used first to set the flow rate by determining the maximum flow rate where near complete (>95%) retention of this sample was reached, while the fractionating laser was set between 7 and 8 W. The subsequent laser powers in regions two and one were determined by first finding the power needed in the first chamber to completely retain the sample exhibiting the highest optical mobility. The determination of the power for the second chamber was determined by finding the power necessary to trap as many individuals from the remaining sample as possible, while trapping a minimal number from the sample with the lowest optical mobility. Once the optimization was complete, several pure injections of each sample were processed and the resulting fractionation efficiencies were analyzed. To complete the experiment, a final mixed sample was injected and the fractionation observed.

Data collection and analysis were performed using IMAGEPRO PLUS version 6.2 (Media Cybernetics, Inc., Silver Spring, MD, USA). Particles were counted manually as the variation in contrast between small (<2  $\mu$ m) particles at different depths was difficult for the automatic identification algorithms to reproducibly achieve.

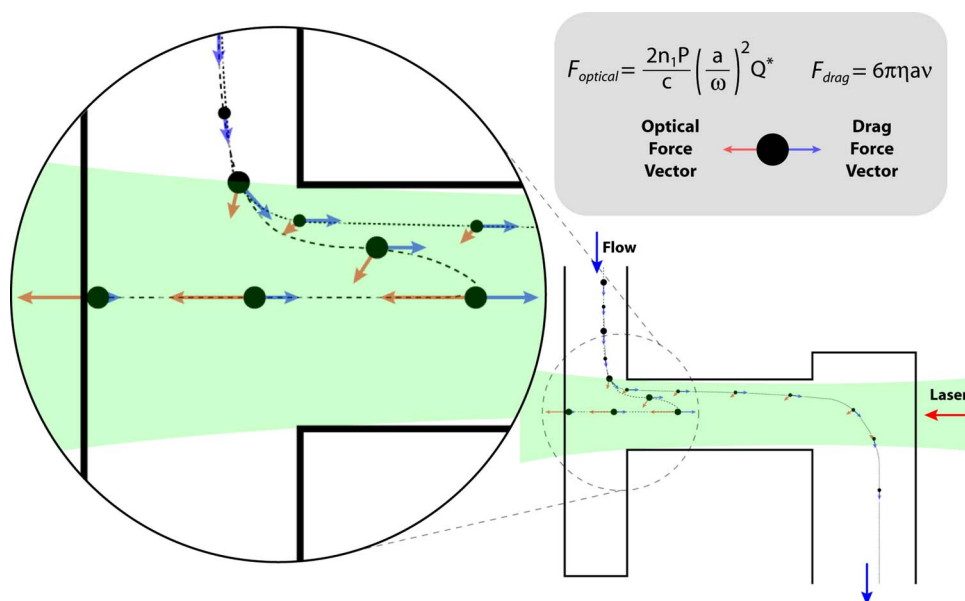


FIG. 2. A schematic of the optofluidic sorting process due to the combined effects of optical pressure and fluid drag. In the magnified region, two particles (different in either size, refractive index, or a combination of the two) initially traveling along the same trajectory are separated and their resulting trajectories with estimated force vectors are shown. Along the centerline, the magnitude of these forces can be calculated using the given equations where  $n_1$  is the refractive index of solution,  $P$  is the laser power,  $c$  is the speed of light,  $a$  is the particle radius,  $\omega$  is the beam radius,  $\eta$  is the viscosity of solution,  $v$  is the linear velocity, and  $Q^*$  is the conversion efficiency of optical pressure transfer. A particle type is retained when the optical force is slightly larger than the opposing fluid drag force, causing it to collect at the wall. Fractionation occurs when, under the same laser and flow conditions, other particle types that have different physical and chemical properties are not retained and flow through the device.

### III. RESULTS AND DISCUSSION

To demonstrate the ability of our system to fractionate multiple entities from a mixed sample, we utilized the device to process two multicomponent samples. Each sample was a three-component mixture with the first consisting of commercially available microspheres of PS, PMMA, and Si. The second involved biological samples of paper mulberry pollen, *B.a.* Sterne strain spores, and *E. coli* cells.

The first mixture included spheres in equal numbers and was designed to be a good simulant in both size and refractive index for the biological separation. For this sample, it was determined that at 7.0 W, a flow rate of 13 nL/min achieved greater than 95% retention for 1.5  $\mu\text{m}$  Si spheres in the third separation region. Following pure injections of the 2  $\mu\text{m}$  PMMA and 10  $\mu\text{m}$  PS, powers of 0.5 and 3.0 W in the first and second separation chambers, respectively, were determined to maximize the fractionation efficiency. As can be seen in Fig. 3, when injected as a mixture, all of the PS beads were retained in the first chamber, while 90% of the injected PMMA beads were retained in the second, and about 80% of the Si beads were retained in the third. Although  $\sim 20\%$  of the injected Si were retained in the second chamber and less than 3% in the first, the high concentrations of PMMA and PS in their corresponding regions represent significant sample fractionation of the entire injected sample.

With the success of the PS, PMMA, and Si simulant samples, a biological mixture containing species likely to exist in an environmental biothreat sample was created. This sample included paper mulberry pollen (12–13  $\mu\text{m}$ ), *B.a.* spores ( $\sim 1.5 \mu\text{m}$ ), and *E. coli* cells ( $\sim 1 \mu\text{m}$ ). For this sample, it was determined that at 8.0 W, a flow rate of 9 nL/min achieved greater than 95% retention of  $\sim 1.0 \mu\text{m}$  *E. coli* cells in the third separation region. Pure injections of the *B.a.* spores and 12–13  $\mu\text{m}$  pollen samples at powers of 1.0 and 3.5 W in the first and second separation chambers, respectively, were determined to maximize the fractionation efficiency. As can be seen

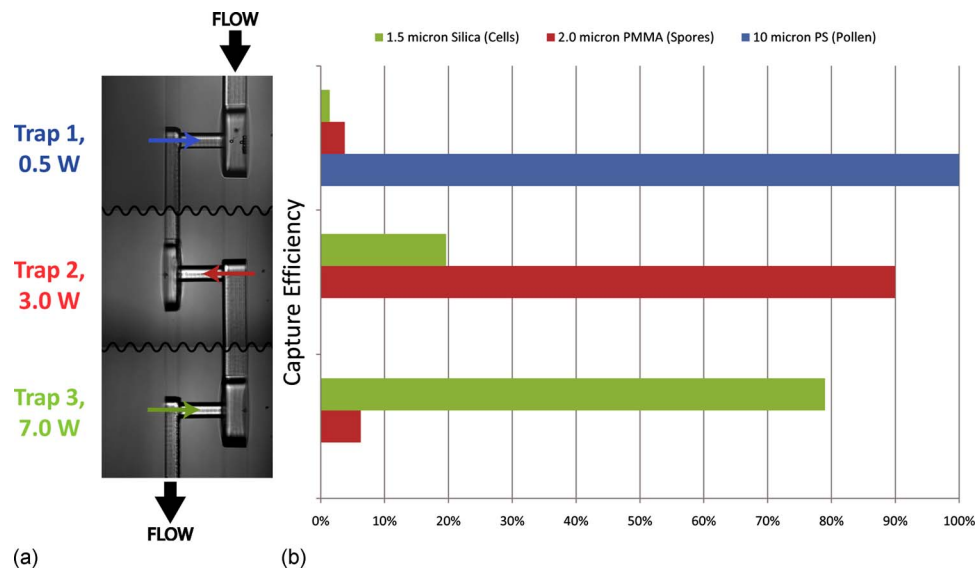


FIG. 3. Fractionation of 10  $\mu\text{m}$  PS, 2  $\mu\text{m}$  PMMA, and 1.5  $\mu\text{m}$  Si diameter microspheres. These beads were selected as approximate surrogates for pollen, bacterial spores, and bacterial cells, respectively. (a) Image showing each retention region, separated by 0.5 mm, and fractionated samples. (b) Graph showing the capture efficiency calculated as the percentage of particles held in each region from the total number of each species injected.

in Fig. 4, all of the pollen was retained in the first chamber while 80%–90% of the injected *B.a.* spores were retained in the second, and 80%–90% of the *E. coli* were retained in the third. Although there was cross contamination of *B.a.* spores and *E. coli*, the concentrations of pollen, *B.a.* spores, and *E. coli* cells in their corresponding regions were greater than 80% and thus achieved significant fractionation relative to the injected mixture sample.

The observed slight contamination by other particles in each experiment can be attributed to the observation that when particles do not enter along a single laminar flow trajectory, as in

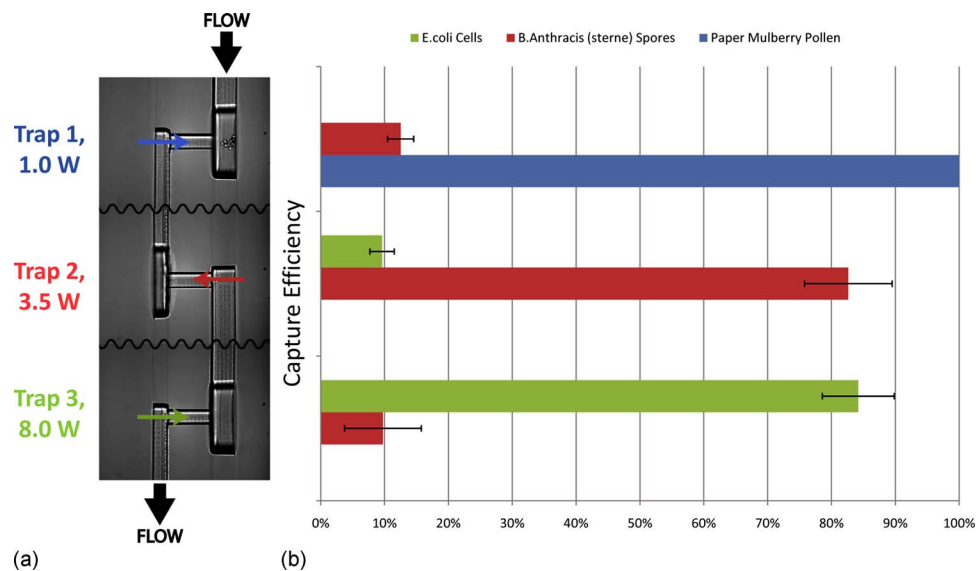


FIG. 4. Fractionation of paper mulberry pollen, *B.a.* spores, and *E. coli* cells. (a) Image showing each retention region, separated by 0.5 mm, and fractionated samples. (b) Graph showing the capture efficiency calculated as the percentage of particles held in each region from the total number of each species injected.

Fig. 2, but enter anywhere in the fluidic channel, particles have a range based on their position in the inlet flow at the turn into the 50  $\mu\text{m}$  channel, where they are retained with the particle they would typically be separated from. The decrease in this entrance range will increase the purity and resolution of future separations.

#### IV. CONCLUSIONS

We have shown that optical chromatography techniques can be combined in series to fractionate a multicomponent biological sample. The design of a complex microfluidic device coupled with novel fluid handling and optics to introduce three independent lasers has allowed us to achieve three-way fractionation within a relatively compact area. This is a significant advance over the single fractionation device that is only able to separate a single population from a multicomponent sample. Further microfluidic designs that incorporate methods to focus samples in the separation regions could greatly increase the performance of this method.

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