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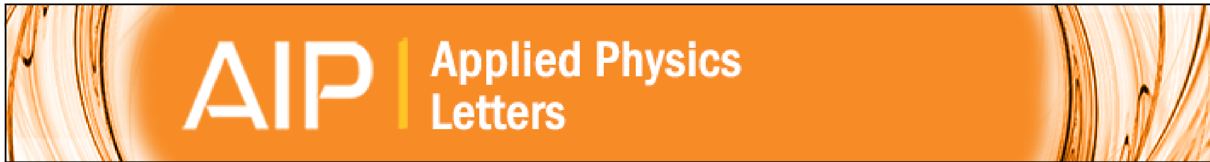

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Optical trapping and fluorescence detection in laminar flow streams

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An optical laser trap with fluorescence excitation/emission capability has been integrated in a flow cytometric geometry for the study of microparticle confinement and off-axis fluorescence detection in laminar flow streams. Measurements of particle escape velocity, trapping efficiency, and fluorescence intensity are presented for 2 μm diameter dye-tagged latex microspheres in laminar flow streams having velocities of up to 12 mm/s. Experimental results are compared with theoretical values for flow velocity and fluorescence intensity and found to be in excellent agreement. © *1995 American Institute of Physics.*

Various applications in molecular biology, biophysics, and biotechnology make use of microparticles as host substrates, or "handles," to which cells or molecules can be attached for manipulation, transport, force measurement, and optical probing.[1] In DNA sequencing, for example, fluorescence-labeled DNA strands are often attached to latex or paramagnetic beads which function as handles that can be held stationary in a flow stream while nucleotide fluorescence from the DNA molecule is acquired.[2–5] The ability to confine micron-size particles in rapid flow streams and subsequently perform optical measurements is therefore essential. From among the available techniques for particle confinement and discrimination, the single-beam gradient force trap[1,6] and flow cytometer[7] have proven to be powerful tools for the optical micromanipulation of single particles and cells in stationary fluids; and the rapid sampling, sorting, and separation of microparticles in flow streams, respectively. While optical traps have also been used in flow cytometric studies,[8] their performance characteristics in flow streams need to be further detailed.

In this letter, we report the simultaneous implementation of infrared optical trapping and off-axis fluorescence emission detection in a laminar flow stream. Using dye-tagged latex microspheres as test particles, and a laser trap for single particle confinement in a flow cytometric geometry, optical trapping efficiencies and fluorescence signal levels are characterized in a square-bore microchamber for values of particle depth, flow velocity, and laser power that might be encountered in, for example, flow cytometer or DNA sequencing experiments. A scanning laser trap is used to displace test particles by more than ±20 μm off-axis, in directions parallel and perpendicular to the flow stream, while particle fluorescence is measured. The parameters necessary to achieve stable trapping, while maintaining high fluorescence signal-to-noise (S/N) ratios (>30 dB), in flow streams having velocities of 12 mm/s, are also described. The results presented herein are relevant to any application that requires noninvasive positioning of samples upstream, or downstream, from the detection probe volume, or characterization of the spatial dependence of optical collection efficiency in flow cytometric fluorescence detection systems.

A schematic of the experimental system is shown in Fig. 1. The flow system consists of a 500 μm×500 μm borosilicate glass, square-bore chamber having a wall thickness of 100 μm. An aqueous solution is pumped through the chamber via a motor-driven, glass syringe that is controlled by a motion controller with a resolution of μm/s. Flow rates could be varied from 0.10 to 50 μl/s when either 100 or 1000 μl syringes were used. The aqueous solution contains a suspension of 1.0 and 2.0 μm diameter latex microspheres (mass density ρ=1.055 g/cm^3) that are labeled with a yellow-green fluorescent dye probe (Molecular Probes) having excitation and emission peak wavelengths at 490 and 530 nm, respectively. To create an optical trap, light from a cw Nd:YAG laser (1064 nm) is passed through a Zeiss IIIR-S microscope

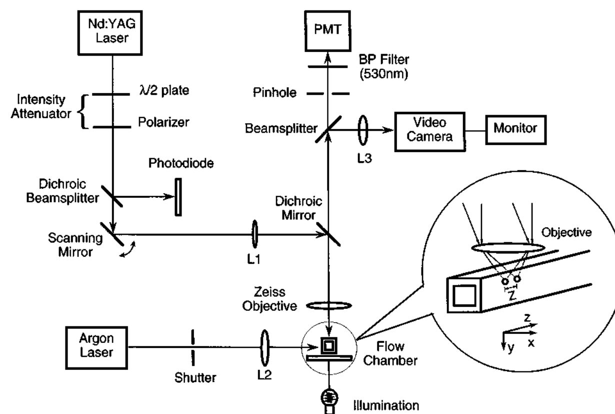

FIG. 1. Schematic diagram of the optical system used for simultaneous optical trapping and fluorescence detection in a flow stream.

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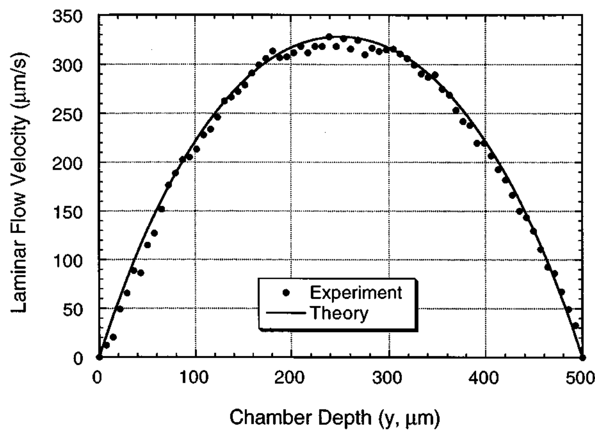

FIG. 2. Laminar flow velocity for a 500 μm wide square-bore microchamber measured experimentally using optical Doppler tomography.

that uses a Zeiss Neofluar 100×(1.3 NA) oil-immersion microscope objective (MO). This objective, having a working distance of ~240 μm, focuses the laser light to its near diffraction-limited spot size ($2\omega_0 \sim 0.8$ μm). The beam is polarized along the flow direction (z axis), while its intensity is controlled with the use of a combined rotating half-wave plate and polarizing prism. Beam power is continuously monitored via the use of a beam splitter and optical photodetector. A motorized scanning mirror, placed in the plane conjugate to the rear pupil of the objective lens, is used to scan the trapping beam about its central axis, in a direction either parallel (z) or perpendicular (x) to the flow (z-axis) direction. A scanning range of more than ±30 μm in either direction could be achieved. At the same time, a cw argon laser (488 nm), focused by an 87 mm focal length lens mounted on a multiaxis translation stage is used to irradiate, and excite fluorescence from, the trapped sample at an angle of 90° with respect to the trapping beam, while fluorescence is collected along the axis parallel to the trapping beam by the same MO used to form the trap. A single MO was used for both trapping and fluorescence collection, based on the space constraints imposed by the chamber design, and the need to trap away from the flow cell wall with high efficiency, while working with a high NA lens and rapid flow velocities. After passing through a 530±3 nm bandpass filter, the fluorescence signal is collected by a photomultiplier tube and acquired by a personal computer. Here, the collection volume for the signal is defined by the combination of the focused Ar^+ laser beam and the spatial filter in the image plane. By minimizing this volume, the background signal from nontrapped beads or other scattering sources can be greatly reduced. In this case, a S/N ratio of >30 dB was obtained when the sample was irradiated with a tightly focused beam and fluorescence collected along a path orthogonal to the excitation path.

To study the effectiveness of the trapping process in a laminar flow stream, a particle was first trapped from a flowing sample stream at a given chamber depth when the flow chamber was centered about the trapping axis. The size of the trapping volume was estimated to be ~100–1000 μm^3 (0.1–1.0 picoliter). The syringe pumping rate, corresponding to the chamber volumetric flow rate (V_0), was gradually increased until the trapped microsphere could no longer be held in the trap. The maximum volumetric flow rate ($V_{0\max}$) for which the sample remained trapped was then recorded for different trapping powers, and the measurements repeated for different chamber depths. For a flow chamber having a rectangular cross section, the laminar flow velocity in the z direction is related to the volumetric flow rate V_0, and is given, in mm/s, by:[9]

$$v(x,y)=\frac{\pi^4 V_0}{4_{ab}}\,\frac{\Sigma_{n=\mathrm{odd}}^{\infty}\Sigma_{m=\mathrm{odd}}^{\infty}\dfrac{\sin(n\pi\bar{x})\sin(m\pi\bar{y})}{nm(\beta^2 n^2+m^2)}}{\Sigma_{n=\mathrm{odd}}^{\infty}\Sigma_{m=\mathrm{odd}}^{\infty}\,[1/n^2m^2(\beta^2n^2+m^2)]}\,, \quad (1)$$

where $\bar{x}=x/a$ and $\bar{y}=y/b$ are dimensionless cross-sectional coordinates, a and b are the chamber width and height, respectively, and $\beta=b/a$ is the chamber aspect ratio. For a measured $V_{0,\max}$, the escape velocity $v(x=a/2,y)$, which is the maximum laminar flow velocity before the particle is lost from the trap, can be determined from Eq. (1). In the present case, for a chamber having $a=500$ μm and $\beta=1$, the laminar flow velocity was measured experimentally using optical Doppler tomography when the volumetric flow rate was 0.039 μl/s,[10] and found to be in excellent agreement with those calculated from Eq. (1), as shown in Fig. 2. The particle escape velocity is dependent on the applied laser power and trapping depth. The laminar flow velocities at different depths were used to determine the optical trapping efficiency Q defined as $Q=Fc/nP$. Here, the applied optical force F is determined from the viscous drag force ($6\pi\eta rv$), where v is the escape velocity, r is the particle radius, η is the fluid viscosity, P is the trap laser power, n is the medium refractive index, and c is the speed of light

The results of trapping 2 μm diameter beads with 100 ×(1.3 NA) and 60×(1.4 NA) objectives are shown in Figs. 3(a) and 3(b), where particle escape velocities are plotted as a function of laser trap power and trapping depth. The results show that the escape velocity is linear in trapping power, and that escape velocities of up to 12 mm/s can be achieved for infrared laser powers of <500 mW. For example, at 30 μm below the chamber surface, a laser power of 200 mW is sufficient to confine a 2 μm bead at flow velocities ranging from ~3 to 4 mm/s. In terms of Q, trapping efficiency is seen to decrease from 0.19 to 0.10 (±0.01) when the trapping depth increases from 6 to 30 μm for the 100× objective, and drop from 0.29 to 0.10 between the depths of 5 and 25 μm for the 60× lens. The decrease in trapping efficiency for the higher NA objective can be attributed to the optical distortion associated with the microchamber wall thickness.[11,12] Both objectives are designed to have minimum distortion when used with a 160 μm thick cover glass, as opposed to the 100 μm thick chamber wall used herein. Even with this limitation, however, microspheres could still be trapped down to a depth of 100 μm, but the trapping effect was observed to be very weak.

Within the same flow chamber, fluorescence measurements were made on trapped samples in a geometry where the excitation and emission optical paths are orthogonal to each other, but the collection path is collinear with the trapping beam, as shown in Fig. 1. Such a geometry is compat-

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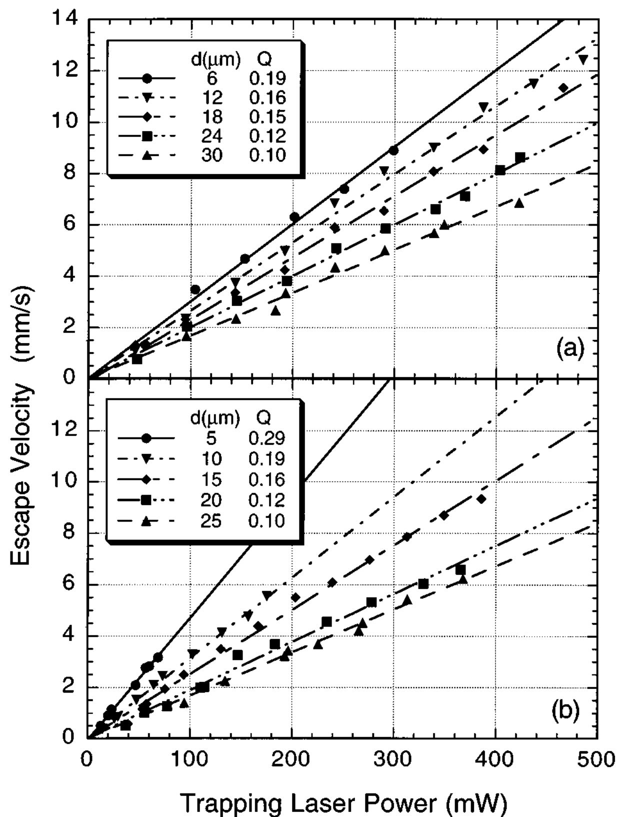

FIG. 3. Dependence of particle escape velocity in a flow stream on trapping power at various trapping depths using (a) 100×, 1.3 NA and (b) 60×, 1.4 NA microscope objectives. Legends display the trapping depth (d) and optical trapping efficiencies (Q), respectively.

ible with the optical geometries typically employed in flow cytometer and single molecule detection schemes.[2–5,13,14] Fluorescence intensity was characterized by displacing the trapped particle off the trapping beam axis in the direction of fluid flow, via beam scanning, and detecting the fluorescence emission signal. The fluorescence quantum yield and probe volume were ~30% and 0.1–1.0 picoliter, respectively. At a depth of 20 μm and an Ar^+ laser power 3 μW, a S/N ratio of >30 dB with micron spatial resolution was achieved. In Fig. 4, the magnitude of the fluorescence signal is shown as a function of particle position, measured with respect to the trapping beam origin (x, $y=a/2$ μm) along the flow direction. The data are fit by a Gaussian function with a $1/e$ half-width of $\omega_0=14.8$ μm. This value precisely corresponds to the diffraction-limited beam width of the Ar^+ excitation beam ($2\omega=2$ mm) when focused by an 87 mm f.l. lens. Here, the ratio of the fluorescence to excitation intensities is constant with respect to the particle position. This result suggests that, using the confocal geometry, the collection of the fluorescence signal from the trapped sample has been optimized, and that fluorescence detection efficiency is not degraded with off-axis sample displacement. This follows from the fact that, for a 100× objective having a 6 mm back aperture and an effective focal length of 1.8 mm, beam scanning of ~±20 μm in the sample plane corresponds to an image shift of less than ±350 μm(<6%) at the back aperture. Hence, the fluorescence signal is predominantly determined by the spatial profile of the excitation beam.

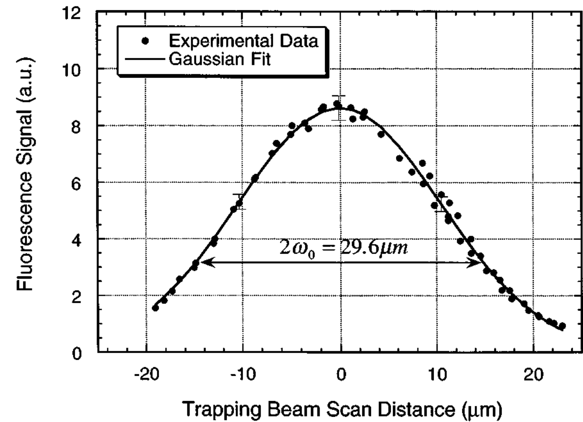

FIG. 4. Fluorescence signal measured as a function of particle displacement off the trapping beam axis, but in the direction of fluid flow. A Gaussian fit of the experimental data yields a $1/e$ half-width of $\omega=14.8$ μm.

In summary, we have examined the processes of optical trapping and fluorescence detection from microspherical particles in laminar flow streams. The results are important to applications which require sample position to be distinct from detection probe volume.

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