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Abstract	We introduce a new approach towards the flow control and detection of colloids in microfluidic specimens. We fabricate hybrid polydimethylsiloxane (PDMS)/glass microfluidic chips equipped with parallel micrometer and sub-micrometer channels with different width and thickness. We image and detect the colloid flow direction through the microchannels by coupling laser-light-scattering in a restricted region of a single channel. We control single polymer colloids by means of a computerized pressure-based flow control system and study the Poiseuille flow through channels with different square cross section. We demonstrate the possibility of in situ sensing populations of colloids with different dimensions down to the sub-100 nm scale.

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Colloid Flow Control in *Microchannels* and Detection by Laser Scattering

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Abstract We introduce a new approach towards the flow control and detection of colloids in microfluidic specimens. We fabricate hybrid polydimethylsiloxane (PDMS)/glass microfluidic chips equipped with parallel micrometer and sub-micrometer channels with different width and thickness. We image and detect the colloid flow direction through the microchannels by coupling laser-light-scattering in a restricted region of a single channel. We control single polymer colloids by means of a computerized pressure-based flow control system and study the Poiseuille flow through channels with different square cross section. We demonstrate the possibility of in situ sensing populations of colloids with different dimensions down to the sub-100 nm scale.

Introduction

Single particle flow control, counting and sizing in fluidic specimens is of paramount importance in environmental, industrial and clinical analysis, on-chip particle synthesis and biological sciences [1]. Micro- and nano-fluidics [2] are emerging technologies that rely on biocompatible and low cost materials and mass production fabrication processes, allow the exploitation of tiny liquid volumes and low analyte concentrations and offer an accurately controllable environment. The most common approach regarding the fabrication of *microfluidic* devices consists of a combination of photo- and soft lithography [3] that generally allows only a 2-dimensional control of the features on a same chip.

Among other technologies for the fabrication of features with variable size on a same chip – such as laser micromachining [4], electron beam and photolithography [5], multilayer soft lithography [6], gray-tone lithography [7], stereolithography [8], solid-object printing [9] and template assisted molding [10], focused ion beam (FIB) has a number of advantages such as high sensitivity and direct fabrication in selective areas without any etch mask. FIB milling has been previously exploited for the fabrication of nanofluidic channels [11] and microfluidic devices [12–14].

On the other hand among other single particle detection approaches – such as Coulter counter with nanocapillaries [15], electrical impedance [16], laser-induced fluorescence [17], particle tracking [18] and correlation spectroscopy [19], laser-light-scattering is a well established detection technique that offers a non-invasive tool for the counting of micro- and nano-particles down to the 100 nm scale such as polymer colloids, blood cells and viruses [20–23].

Here we introduce a novel approach toward the control of single sub-micrometer colloids. We fabricate microfluidic devices equipped with parallel channels with different cross section by exploiting *Platinum* (*Pt*) wires deposited via FIB as templates for soft lithography. We characterize translocations of single particles with size in the range 50–450 nm in terms of event frequency, duration and amplitude by coupling laser scattering in a single channel. *We use channels with different cross section on the same chip to investigate the pressure-driven transport of single polymer colloids with diameter of* 300 *nm*. We demonstrate the in situ sensing of populations of colloids with diameters between 50 and 450 nm.

Experimental

Preparation of Colloidal Suspensions

As test particles for our setup we used polystyrene (PS) nanospheres with mean diameter (52 ± 8) nm and (457 ± 11) nm (Polysciences, Inc. Warrington, PA) in a 2.67% and

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2.63% solids (w/v) aqueous suspension, respectively. In addition poly(methyl methacrylate) (PMMA) nanospheres *with mean diameter of* (290±55) are synthesized by means of emulsion polymerization [24, 25] and dispersed in a 2.63% solids (w/v) aqueous suspension. The saline buffer for the colloidal suspensions is a KCl solution with molarity in the range 5–50 mM.

Chip Fabrication

The fabrication of the microfluidic chip consists of three steps: [26] (*a*) *the deposition of Pt wires on a Silicon substrate via FIB followed by* (*b*) *the patterning of a photoresist layer via photolithography for the realization of a reusable mold;* (*c*) *the replica molding of the latter in PDMS and the chemical bonding on a glass substrate via oxygen plasma functionalization for the fabrication of the final disposable device*. The FIB assisted deposition of the Pt wires is carried out with a Cross-beam 1540 FIB/SEM system (Zeiss, Oberkochen, Germany) equipped with a Ga+beam. A typical Pt deposition is carried out by using an accelerating voltage of 30 kV and a beam current of 100 pA. The scanning frequencies are 20,000 and 200 Hz along the longitudinal and orthogonal wire axis, respectively. For the fabrication of the mold, a layer of AZ 9260 (Microchemicals GmbH, Ulm, Germany) is deposited via spin coating (2,000 rpm for 30 s) on the silicon print master previously cleaned by sonication in acetone and isopropyl alcohol. After a 3 min pre-bake step at 115°C to remove the residual solvent, the sample is exposed to UV light (365–405 nm, 52 mW/cm^2) through a quartz mask (Photodata Ltd, Hitchin, UK) selectively coated with a thin Chromium film *patterned with two symmetrical stirrup shapes separated by a* 18 µm *gap* (Fig. 1a) and ending with four 2 mm-side square pads. Sample and mask are carefully aligned through a MJB4 mask aligner (Karl Suss, Garching, Germany) in a way that the central region of the wire array is positioned under the 18 µm-gap on the mask (Fig. 1b). The sample is exposed for 10 s in hard contact mode (by realizing a vacuum around 0.8 Bar between sample and mask), developed in a deionized water solution of AZ 400k developer (4:1 in volume) for 8 min at steps of 2 min each and finally rinsed with deionized water and dried with nitrogen. The thickness of the obtained photoresist structures deposited over the Pt wires (Fig. 1a) is around 12 µm as measured by a Dektak stylus profilometer (Veeco, Plainview, NY). The sample is baked at 60°C for 3 h and left in air overnight to allow complete evaporation of the solvent.

Replica molding of the device is realized by casting on it a 9:1 (base:curing agent) PDMS mixture and in situ curing at 60°C for 40 min in oven. A typical device is shown in the SEM micrograph of Fig. 1c with the inlet and outlet reservoirs separated by a 18 µm-wide and 12 µm-thick PDMS wall and connected through three hollow channels

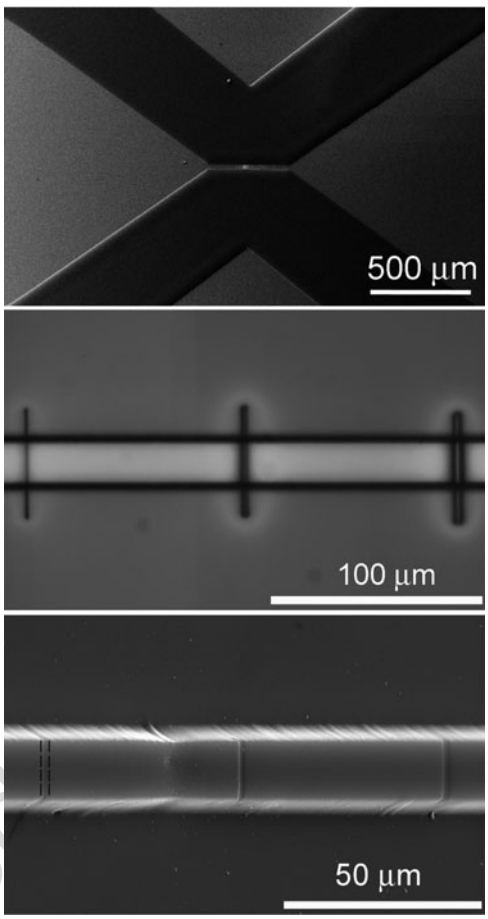

Fig. 1 (**a**) Optical micrograph of the mold: two 12 µm-*thick symmetrical* stirrups made of AZ 9260 are separated by a 18 µm gap. (**b**) Particular of the 18 µm-*long window* of uncoated Si and Pt wires with square cross section of 1, 2 and 3 µm^2. (**c**) SEM micrograph of the resulting PDMS negative replica (tilted at an angle of 38° with respect to the SEM beam column) with hollow channels connecting the inlet and outlet reservoir chambers of the microfluidic chip. *Dashed lines* mark the smallest channel

with square cross section of 1, 2 and 9 µm^2 (from left to right, respectively). Four 1.5 mm-wide circular holes are drilled by a 1.5 mm-wide circular disposable biopsy punch (Kai Industries Co. Ltd., Seki City, Japan) in correspondence of the four square pads to enable fluidic access to the microchannels. PDMS is bonded to a glass slide by exposing both surfaces to oxygen plasma treatment (8.5 s exposure to 2.5 W plasma power, Plasma etcher, Diener, Royal Oak, MI). 1.6 mm-wide PEEK tubing (Kinesis, St Neots, UK) is integrated in the holes exploiting the PDMS flexibility thus ensuring tight and fully sealed connections. The device is completed by the connection to external PEEK shut off valves (0.020″ thru-hole, 1/16″ Fittings, Kinesis) on their turn connected to a computerized pressure-based flow control system (maximum applied pressure 75 mbar, sub-mbar pressure steps MFCS-4C, Fluigent, Paris, France) that allows to stop and accurately regulate the flow in the microfluidic chip. The pressure gradient is defined as positive when the pressure applied to the inlet is higher than the

one applied to the outlet. In the same way the translocation frequency through the channels is defined as positive when the colloids flow from the inlet to the outlet.

Detection Set-up

Details about the laser scattering detection set-up are reported elsewhere [26]. Briefly a red laser beam is coupled into an oil immersion objective and thus focused in a single microchannel. The scattered laser-light is coupled to a four quadrant photodiode, the voltage signal is amplified and digitized. Pressure-driven translocations of colloids appear as increases in the voltage trace and are isolated by using a custom-made program (LabVIEW 8.6, National Instruments). Specifically the background signal or baseline is calculated every 1,000 points of the trace. The translocation events are recorded when a consecutive number of points (i.e. 40) exceed twice the value of the baseline standard deviation. Translocations of 300 and 50 nm particles through a microchannel with square cross section of 1.4 μm^2 are reported in Fig. 2a and b, respectively. Each single isolated event is fitted by a Gaussian curve (solid lines in Fig. 2c and d, respectively) which allows determination of the duration and amplitude of the translocation event (horizontal and vertical arrows, respectively, in Fig. 2b and d), the time difference with the previous event and the *signal/noise (S/N) ratio. It is noteworthy to observe that the average measured signal/noise ratio and the time difference between two successive events decreases from* 60 *to* 4 *and from* 350 *to* 50 ms*, respectively, for particle diameters of* 300 *and* 50 nm*. The lower S/N is due to the decreased amplitude in the signal that is reflected back into the objective from the smaller particle surface, while the shorter interval within two successive events is due to the presence of a higher number of particles,* 10^{11} *and* 4×10^8 cm^{-3} *for* 50 *and* 300 nm *populations, respectively.*

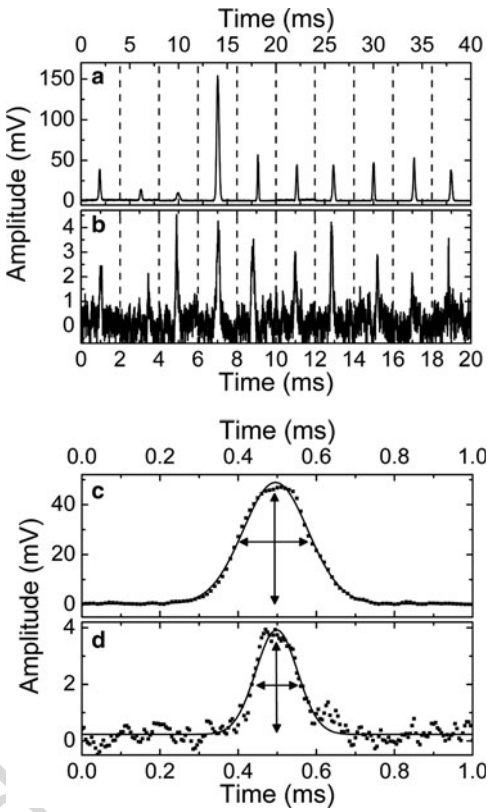

Fig. 2 *Selected intervals of* 4 (**a**) *and* 2 ms (**b**) *showing ten single events isolated from the voltage traces of* 300 (**a**) *and* 50 nm *colloids* (**b**) *translocating a microchannel with square cross section of* 1.4 μm^2. Measured signal of a single event (*squares*) fitted by a Gaussian curve (*solid lines*) with the estimation of translocation duration and amplitude (*horizontal* and *vertical arrows*) for 300 nm (**c**) and 50 nm (**d**) colloids

Results

We investigated the pressure-driven colloid transport through channels with different square cross section. We carried out experiments on two different devices each equipped with an array of three channels with cross section of 0.4, 1.4, 3.2 μm^2 and 1, 4, 9 μm^2, respectively. We measure *the translocation frequency of* 300 nm *particles* with respect to the channel cross section (Table 1) under an applied pressure gradient of 40 mbar. *The general description of hydrodynamic phenomena by the Navier-Stokes equation reduces in most of the microfluidic systems to the linear Stokes equation and the so called Stokes or creeping flow since in the limit of low Reynolds numbers the non-linear term can be neglected* [27]. *In particular the particle flow in closed channel systems can be described by introducing the channel and particle Reynolds numbers* (Table 1), Re_c and Re_p: [28]

$$\mathrm{Re}_c = \frac{U_m \sqrt{S} \rho}{\eta}, \ \mathrm{Re}_p = \frac{U_m d^2 \rho}{\eta \sqrt{S}} \qquad (1)$$

where U_m *is the maximum velocity of the channel flow, S is the channel cross section, d is the particle diameter,* η *and* ρ *the dynamic viscosity and density of the flowing solution. The small values of the channel Reynolds number* (Table 1) *indicate that the non-linear term is negligible*. In particular the pressure-driven, steady-state flow through long, straight and rigid microchannels with square cross section can be described by the Hagen-Poiseuille flow that predicts a second power law of the volumetric flow rate, Q, with respect to S: [27]

$$Q \approx 0.27 \frac{\Delta p}{12 \eta L} S^2 \qquad (2)$$

t1.1 **Table 1** Microchannel cross section S, measured f_m and predicted f_p translocation frequencies and corresponding errors. The error in S is evaluated by considering a 100 nm uncertainty in the SEM measurement of the channel width and height. The errors in f_m is the standard deviation calculated by averaging over measurements acquired for an interval of 30 s. For the error in f_p we take into account the error in S and a ~100 nm uncertainty in the diameter of the laser spot. The values refer to experiments with 300 nm particles

t1.2	S (μm^2)	Re_c	Re_p	ω (s^{-1})	f_m (s)	f_p (s)
t1.3	0.4±0.1	0.001	$3.1*10^{-4}$	$2*10^3$	0.5±0.1	0.3±0.2
t1.4	1±0.2	0.007	$6.1*10^{-4}$	$3*10^3$	0.9±0.3	2.5 ±1
t1.5	1.4±0.2	0.012	$7.7*10^{-4}$	$4*10^3$	3 ±0.4	4.3±1.6
t1.6	3.2±0.4	0.040	$1.1*10^{-3}$	$6*10^3$	5.2±0.6	6.5 ±2.2
t1.7	4±0.4	0.055	$1.2*10^{-3}$	$7*10^3$	8.6±1	7.2 ±2.4
t1.8	9±0.6	0.184	$1.8*10^{-3}$	10^4	9.3±0.9	10.8±3.4

where L is the channel length and Δp is the pressure gradient. For a square cross section the error of this approximate result is around 13% [27]. *At the connection between the reservoirs and the microchannels the Poiseuille description is still approximately correct since Re_c remains ≤ 1 which means that the non-linear term in the Navier-Stokes equation has a vanishingly small contribution and that the inertia effect at the microchannel inlet are negligible. Moreover the laser detection was coupled in the central part of each microchannel far away from the channel inlet and outlet. For particles dispersed in low concentration dispersion, particle flow is described by the fluid flow. In fact, since the particle Reynolds number* (Table 1) *is small particle flow is dominated by viscous drag of the fluid* [27, 28]. Therefore the predicted translocation frequency can be described as:

$$f_p = nQ = n\frac{\Delta p}{12\eta L}0.37S^2 \qquad (3)$$

where n is the number of particles per unit volume, which is estimated to be 4.2×10^8 cm^{-3}.

Moreover inertial effects such as the lift and drag force play a negligible role and the flow remains laminar [28, 29]. *It is noteworthy to observe that the flowing particles rotate following the fluid vorticity* [30]. *In fact in a Newtionian fluid in shear flow with no-slip boundary conditions imposed on the surface of the sphere, the rotation speed of a single particle, ω, is given by:* [30]

$$\omega = \frac{\dot{\gamma}}{2} \qquad (4)$$

where $\dot{\gamma}$ is the shear rate [31]. *Typical rotation speed are reported in* Table 1. *No shear thickening* [32] *is expected since the suspended colloids occupy only a volume fraction down to* 10^{-6}.

For an applied pressure gradient of Δp=40 mbar and taking into account that for large channels ($S>1.1$ μm^2) the laser spot, a, occupies only a fraction of the microchannel volume:

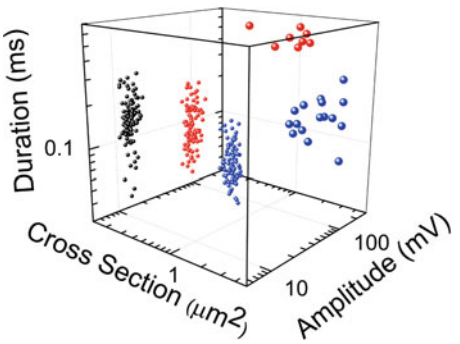

Fig. 3 3D scatter plot reporting duration and amplitude of 100 translocations of 50 and 450 nm colloids (*small* and *large spheres*, respectively) through channels with square cross section of 0.4, 1.4, 3.2 μm^2 (*black*, *red* and *blue spheres*, respectively)

$$f_p = 2.86S^2 \ \text{Hz}/\mu\text{m}^4 \text{ for S1.1 } \mu\text{m}^2$$

$$f_p = 2.86\sqrt{S}\frac{4}{3}\pi a^3 \ \text{Hz}/\mu\text{m}^4 \text{ for S1.1 } \mu\text{m}^2 \qquad (5)$$

The translocation frequency, f_p, predicted by (5) reproduces the measured translocation frequency, f_m, within the error bars (Table 1).

Therefore the laser scattering set-up coupled into a single channel provides quantitative information about the transport of particles with diameters of a few hundreds of nanometers. Moreover the presented platform allows the sensing of particles with diameter down to the 50 nm *scale*. In particular the sensing of particles over a range of diameters is easily achieved by exploiting channels with different cross section on the same microfluidic chip. As a proof of concept 50 nm particles are initially injected in the chip and detected in three different channels with cross section 0.4, 1.4, 3.2 μm^2. Thereafter a small amount of 450 nm particles (around 1:100 w/w ratio with respect to the 50 nm ones) is injected and the translocations of both types of particles are recorded. The biggest particles reach the outlet reservoir by going through the medium and the biggest channels (red and blue large spheres) but do not travel across the smallest channel as highlighted in the scatter plot in Fig. 3. In fact both small (amplitude$<$10 mV) and big particles (amplitude$>$30 mV) are detected in the two former channels while only small particles (black small spheres) are detected in the latter one. Therefore by simply looking at the scattering events in different channels in the same chip one can easily detect populations of particles over a range of diameters.

The presented novel microfluidic platform can be readily exploited to investigate the interactions between the flowing particles and the device surface by studying the transport

parameters (i.e. event frequency, amplitude, duration) as a function of the salt concentration. *We are currently exploring the possibility of employing such microfluidic systems to mimic the diffusion of metabolites across membrane protein pores and to investigate and model the physics of single channel transport. Further tuning of the glass/PDMS surfaces through polymer or protein coating may be required for more specific biological applications such as the investigation of DNA translocations under concentration/pH gradient or electro-phoretic/osmosis force* [15]. The exploitation of stiffer material extensively used in soft lithography for the generation of 50 nm features [33] could open the way for the realization of nanochannels for nanofluidics *while the improvement of the detection set-up for example with the integration of a high speed nanoscanning piezostage could allow the investigation of the transport of particles with diameter down to the sub-*50 nm *scale*.

Conclusions

We have proposed a simple and versatile approach for the control of sub-micrometer colloids in polymer-based lab-on-a-chip systems equipped with arrays of parallel channels with different square cross section down to 0.4 μm^2. We have coupled laser scattering in single microchannels for the in situ detection of single translocating colloids with minimum detectable particle size of 50 nm. We demonstrate that the pressure-driven transport of 300 nm particles through channels with different cross section can be modeled by a Poiseuille flow. Finally we demonstrated the sensing of particles with different diameters by exploiting channels with a range of cross sections on the same microfluidic chip.

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