VISUALISATION OF SINGLE SUB-MICRON PARTICLES BY LIGHT SCATTERING IN A MICROFLOW

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ABSTRACT

We describe the fabrication and operation of microfluidic chips for optical visualization of individual sub-micron and nanoscopic particles by light scattering in a continuous flow. Particles beyond the resolution limit of an optical microscope can be directly seen and recorded as they scatter the incident laser light, which permits label-free detection of single particles. Microchannels of 120 μm height structured in Ordyl dry film resist on the chips were traversed by a laser beam coupled in by a glass fiber so that particles in the flow cross the beam and scatter a portion of the incident light. At an angle of 90° to the laser beam the scattered light is observed via a microscope and recorded with a camera. 250 nm particles appear as clearly visible bright spots of several micrometer diameter on the video and can individually be counted. Hydrodynamic focusing of particles with a sheath flow was performed in two ways. Vertical focusing was done in order to confine the particle containing sample liquid to the bottom of the chip so that all particles were approximately in the focal plane of the microscope and hence circular fringes could be avoided. By using lateral focusing the sample stream was confined to the middle of the channel, which leads to a smaller detection region and allows the use of higher microscope magnification. With the proposed sensor setup and microfluidic chips it is possible to visualize and count suspended particles.

1. INTRODUCTION

The detection and characterization of micro- and nanoparticles in fluids plays an important role in many industrial, environmental, and scientific areas. Light scattering principles, e.g., dynamic light scattering (DLS), are often applied for purposes like counting or sizing particles suspended in liquids [1]. Furthermore, microfluidic device designs have been presented that combine microfluidic operation and light scattering functionality [2-6]. For instance, the characterization of sub-micron particle ensembles with [2] and without flow [3,4], as well as the detection of particles in the micrometer size [5,6] was done. Still, these methods do not offer the possibility of label-free individual sub-micrometer sized particle detection in a microfluidic channel with continuous flow. On the other hand, principles like nanoparticle tracking analysis are applied for measuring the size of single nanoparticles only in a non-moving liquid [7] and particle image velocimetry (PIV) usually relies on tracking of fluorescent particles typically in the range of 0.2 to 1 μm [8]. Flow cytometers are typically employed for characterization of cells limited to the low micrometer regime [9] and only few studies have dealt with light scattering detection of submicron sized sample, e.g., like viruses [10].
Here we describe the fabrication and operation of a microfluidic particle sensor setup which allows visualization of single sub-micron particles beyond the optical resolution limit of light microscopes in a continuous flow combined with capabilities to confine particles by hydrodynamic focusing.

2. MATERIALS AND METHODS

2.1 Microfluidic chip design

The microfluidic chips basically consist of channel structures made of negative dry film resist sandwiched between a glass and a PMMA (polymethylmetacrylat) substrate with inlet holes. Glass fibers can be inserted into grooves at the chip edges to couple laser light into the chip. Light scattered by particles flowing through the channels can be observed from the top with a microscope and camera setup.

An easy insertion of glass fibers into fiber grooves is possible since the bottom PMMA part is protruded over the glass chip and can therefore serve as a guidance in one direction (Fig. 1a). This requires that the channel structures are fabricated on a glass wafer which is subsequently diced into single chips before they were individually bonded to larger PMMA slides. Dry film resist was used due to its easy handling and suitability for fast and inexpensive fabrication of micron sized structures. A channel height of 120 μm and channel widths of 200 to 300 μm turned out to be convenient for handling and particle detection.

Two chip designs were realized and utilized for particle light scattering experiments. The first design comprises a single channel with two inlets and one outlet, whereas one inlet serves as sample inlet while the other is used for the sheath liquid. By flowing the sheath liquid above the sample liquid from the backside it is possible to confine the particle sample to the bottom of the chip (Fig. 1b), hence all particles are concentrated in the focal plane of the microscope. In this mode of operation, the scattered light from the particles appears as bright spots of several micrometer in diameter, while particles out of focus would show additional ring-shaped patterns that could overlap each other and complicate particle visualization. In the second design the sheath flow constrains the sample from both sides to the middle of the channel (Fig. 1c). With this method the width of the sample flow with particles is reduced allowing a higher microscope magnification and hence a better resolution. Laterally, the particle velocity distribution is therefore approximately uniform and particles will not get in contact with the side walls.

![Figure 1: Schematics of the microfluidic devices for particle detection by light scattering: a) Basic structure of the chips. Laser light coupled into the channel is scattered by particles and recorded with a microscope and digital camera from the top; b) Principle operation of vertical sample focusing and c) lateral sample focusing.](image-url)
2.2 Fabrication

Four layers of Ordyl SY330 dry film resist (Elga Europe, Italy) with thicknesses of 30 μm were laminated on a 500 μm thick glass wafer with a standard office laminator at a temperature of 105°C in order to create a designed film height of 120 μm (Fig. 2a). Structures were defined by UV-lithography (mask aligner MA 150 M, Karl Suss, Germany) with a foil mask (Zitzmann, Germany) at 26 s exposure time (Fig. 2b).

After a post exposure bake at 85°C for 1 min (Fig. 2c), the resist was developed under ultrasonic agitation and manual rotating of the wafer to ensure an even development of the structures. A commercial Ordyl developer consisting of 56wt% xylene, 30wt% 2-butoxyethylester and 14wt% ethylbenzene (Sigma-Aldrich, USA) was used. After one minute the wafer was removed from the developer and thoroughly rinsed with isopropanol and deionized water (Fig. 2d).

In order to saw the glass wafer with the open Ordyl channel structures into single dies, the wafer was covered with protective film (AZ 6612 photoresist) to ensure that the Ordyl structures were not damaged and diced with a diamond saw blade. The photoresist was stripped from each die by spraying with acetone and immediate rinsing with isopropanol to avoid damaging of the dry film resist structures by too long exposure to acetone solvent. The dies were individually bonded to small PMMA plates with drilled inlet holes. Bonding was done at 85°C for 30 min by applying pressure on the aligned chip which was clamped between two metal plates in a mechanical press (Fig. 2e). Curing of the resist structures was done without pressure at 85°C for two hours until the Ordyl resist appeared almost fully transparent (Fig. 2f). Photographs of two exemplary chips are shown in Fig. 3.
2.3 Sample preparation

250 nm iron oxide microspheres with gold surface suspended in an aqueous solution were purchased from micromod, Germany. The initial concentration according to the specifications was 25 mg/mL or $5.7 \times 10^{11}$ particles/mL. Prior to experiments, the particle suspension was diluted with filtered DI water at a ratio of 1:10⁴ to reduce the particle concentration to $5.7 \times 10^7$ particles/mL. The sample was ultrasonicated for 5 minutes and subsequently shaken with a vortexer. The microfluidic devices and tubings were flushed with filtered DI water and isopropanol to remove contaminations before the experiments. Filtered DI water was also used as sheath flow liquid.

2.4 Sensor setup

Syringe pumps (Cetoni Nemesis) and 250 μL glass syringes (Hamilton) were used to pump sample and sheath liquids through the microfluidic chips. Fluidic interconnections were glued to the bottom PMMA part of the devices. A green laser module (Laser Roither) with a wavelength of $\lambda = 532$ nm and nominal output power of $P_{\text{out}} = 20$ mW was attached to a fiber collimator and a glass fiber with 90 μm core diameter. The glass fiber was inserted into a fiber groove on the chip to couple in laser light into the channel. A microscope (Zeiss) with an attached digital camera was used to observe and record the 90° scattered light from the top. Recorded videos were subsequently analyzed with MATLAB software.

2.5 Data processing

Apart from the scattered light from the particles there is also a considerable contribution to the total scattered light by the chip itself. Since this stray light generated in the chip is constant, it is easily possible to minimize it and enhance particle scatter light visibility. One potential method of processing the recorded videos with MATLAB is subtracting the background. Due to slight drifting and temporal variation of laser light intensity it is not useful to capture only one reference video frame in absence of particles and subtract it from the following recording. However, a reference frame can be taken at predefined time steps (e.g. every 30 frames) and used for background subtraction. If particles are present in this frame, the signal will be subtracted at their actual position which can result in obscuring another particle transiting this position. Since this will only occur for a short instant the effect is negligible, especially when the particle concentration is low. Another video processing method consists in creating the difference of two successive video frames. Thus static signals are cancelled out and only those signals due to particle movement remain. In that case drifting of laser intensity is irrelevant. Both methods provide good particle visibility in the processed videos. Additional low-pass filtering can be used to remove short-time fluctuations and smoothen out the particle images.

3. RESULTS

3.1 Particle visualization

A fluid sample with 250 nm particles was pumped through the microchannel at flow rates around 100 nL/min without vertical or lateral hydrodynamic focusing. The laser beam which is collimated by an air/resist lens illuminates the sample perpendicular to the flow direction (Fig. 4). Since the laser beam is only collimated in the plane of the chip it covers the whole height of the channel. With 65-fold magnification of the microscope the field of view in the detection region is approximately 280 μm x 160 μm. On-chip fiber grooves and corresponding air/resist lenses for collimation can be arranged in different angles than perpendicular to the channel if desired. It would also be possible to insert one or more fibers to attach further laser sources or photodetectors.

Particles crossing the laser beam scatter laser light which can be observed with the microscope and recorded with the camera. When a particle is in the focus of the microscope it appears as a bright dot which is a few micrometers in diameter while particles outside the focus show additional concentric rings. If the distance between the microscope focal plane and the particle increases, the rings increase in diameter and further rings become visible. At the same time, the intensity of the centre spot reduces. For very large distances between the focal plane and the particle (>50 μm) only the outmost ring is visible. Fig. 5 shows recorded original frames as well as data that was processed by frame differencing method to enhance particle visibility.
Figure 4: Top view of the detection area and the laser beam coupled into the chip from the side. An air-resist lens is used to collimate the beam. The glass fiber inserted into the fiber groove is 90 μm in diameter, the channel of the depicted device is 200 μm wide and 120 μm high. An additional lens with corresponding fiber groove at a different angle can be seen in the lower part of the figure.

Figure 5: Images of the scattered light in the detection region. a) Frame of a recorded video before processing, two particles (250 nm diameter) are marked in the image. Particles are scarcely visible due to substantial stray light stemming from the chip structures. The channel walls are illustrated by the white dashed lines; b) Difference image of the same frame, i.e., the previous video frame was subtracted in order to remove the constant part of the scattered light and to get only the signal due to moving particles; c) Five-fold enlarged image of the dotted region. Particles are slightly out of microscope focus and exhibit additional concentric rings.

3.2 Vertical particle focusing

When particles are present across the whole channel height their scattering images can overlap which would complicate further image processing. To simplify the digital acquisition of particle scattering events, it is advantageous to obtain particle images that resemble single spots instead of complex patterns.

In the vertical particle focusing mode the sample liquid is pushed to the bottom of the channel by a sheath flow so that all particles can be in the focal plane of the microscope (Fig. 6). With sheath and sample flow rates of 100 nL/min and 50 nL/min, respectively, particles were confined to the lower quarter of the channel.
channel and appear as single spots. When the microscope focus is not in this region, typical annular patterns occur. Though particles are constrained to a narrow volume of the channel, they can still be present over the whole width of the channel and travel at different velocities due to the parabolic flow profile. Also, it is possible that particles in this size regime diffuse distances of several μm in the out-of-plane direction which can cause minor changes in the appearance of their scattering pattern. Diffusion of particles from the sample stream to the sheath flow was considered to be negligible.

3.3 Lateral particle focusing

Lateral focusing of particles was performed at a sample flow rate of 30 nL/min and sheath flow rates of 50 nL/min. Fig. 7a shows how a dyed sample stream is squeezed by the sheath flow from left and right at the channel junction. Particle scattering images (Fig. 7b) show that particles are confined to the middle of the channel and recorded scattered light was observed. Only particles which were approximately in the focal plane of the microscope appear as single spots, while particles out of focus show additional rings or are visible only as rings.
channel. Due to the parabolic flow profile in the out-of-plane direction, the particles can travel at different velocities. Owing to their high flow velocity and the limited frame rate of the camera (50 frames/s) particles may appear blurred and occasionally stretched. Also, with this approach particles in the sample flow can still pass the detection area at different channel heights which results in a variety of scattering patterns that can possibly overlap. Additional sheathing or lifting of the sample stream would constrain the particles to a small region at the top or bottom of the channel. By a combination of both the sample could be focused in the middle of the channel [9].

![Figure 7: Lateral focusing of a sample stream. a) Micrograph of a dyed sample that was confined to the middle of the measurement channel by two sheath flows; b) Images of two particles constrained to the middle of the channel. The velocity of the particles is different due to the parabolic flow profile across the channel height. Four subsequent frames are depicted in time steps of 60 ms, channel walls are illustrated by dashed lines.]

4. CONCLUSIONS

Low-cost microfluidic devices were fabricated in a rapid and simple way using Ordyl dry film resist and standard lithography. Laser light scattered by 250 nm diameter particles flowing through the detection volume of the chip can be observed and recorded. By processing the videos it is possible to substantially reduce the light scattered from chip structures and to enhance the signal to noise ratio. Hydrodynamic focusing of the sample stream in the out-of-plane direction permits a better visualization of particle scattering events and simplifies the determination of the particle number by analyzing the recorded videos. A combination with lateral focusing would provide a smaller measurement area and allow the use of a higher microscope magnification. With a high speed camera the detection and counting of scattering particles at larger flow velocities would become possible, but usually also at the cost of image resolution. Also, further glass fibers can be inserted into the device to use additional laser sources or photodiodes. To obtain a good signal from even smaller particles a laser with higher output power or a more efficient fiber collimator system would be necessary, since the scattering cross section of the particles reduces rapidly with their size.

The proposed method allows counting of the total number of particles in previously size-fractionated particle suspensions to determine the particle size distribution (PSD). It further offers the potential to perform continuous on-line submicron and nanoparticle monitoring in liquids or gases without the need for fluorescent labeling. Size determination of unknown particles is feasible by utilizing scatter intensity or Brownian motion of particles that can be inferred from the recorded video signal.

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REFERENCES AND CITATIONS


