Fiber free plug and play on-chip scattering cytometer module – for implementation in microfluidic point of care devices

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FIBER FREE PLUG AND PLAY ON-CHIP SCATTERING CYTOMETER MODULE
- FOR IMPLEMENTATION IN MICROFLUIDIC POINT OF CARE DEVICES

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ABSTRACT

In this paper, we report on recent progress toward the development of a plug and play on-chip cytometer based on light scattering. By developing a device that does not depend on the critical alignment and cumbersome handling of fragile optical fibers, we approach a device that is suitable for non-expert users and Point-Of-Care (POC) applications. It has been demonstrated that this device is capable of detecting and counting particles down to 1 µm at 100 particles per second. This device only depends on a single microfluidic channel. Hence, the device is easy to implement, or to use on its own.

KEYWORDS: Cytometry, Microfluidic, Optical, Scattering, Plug-and-play, Particles

INTRODUCTION

A rapid detection of a large quantity of individual unlabeled micrometer sized particles is a daunting task for most bio-chemical applications including commercial cytometers. We present a polymer-based (SU-8, PDMS) on-chip flow cytometer, which can be implemented in many other microfluidic devices, supporting detection of particles ranging from, at present, 1-10 µm (Figure 1). The cytometer is based on scattering, which eliminates any requirements for fluorescent labels and other markers. By implementing this on-chip cytometer module, tedious sample handling is avoided, and test sequences become faster, cheaper and less prone to human error. Our ambition is to create a much cheaper alternative to commercial cytometers that is easy to handle and small enough to be used in field laboratories. This device distinctively differs from other on-chip cytometers (such as our own previous device [1] and that of Lee's group [2]) by not depending on optical fibers. Instead, this device is based on a combination of bulk lenses and tailor designed on-chip lenses as well as a simple pin-aided alignment system, which enables true plug and play operation.

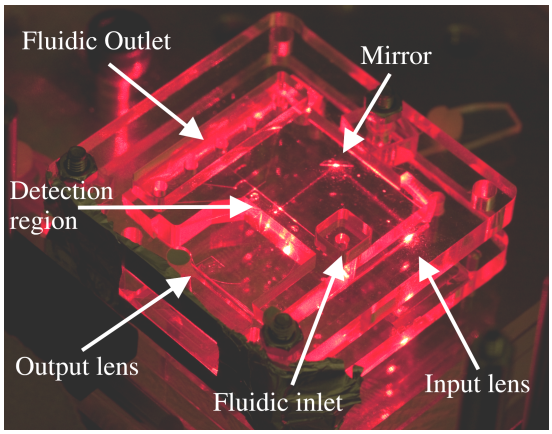

Figure 1: A photograph of the plug and play device, here shown without fluidic connections. The laser beam enters through the input lens in the lower right corner. After crossing the fluidic channel, the scattered light exits the chip through the output lens in the lower left corner.

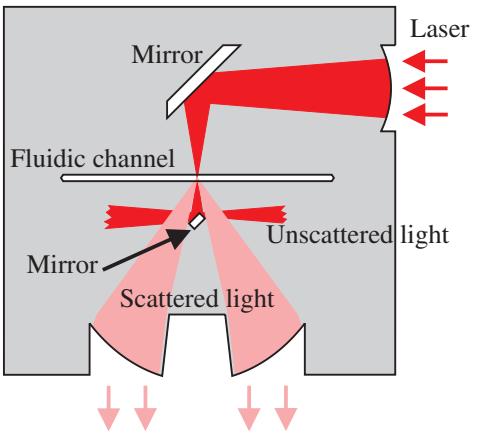

Figure 2: A sketch of the optical part of the chip. The SU-8 layer (grey) acts as one big planar waveguide. Non-scattered light is separated from the scattered light using an SU-8 mirror. For illustrative purposes objects are not to scale with respect to each other.

THEORY

The 5 mW 635 nm incident laser beam is first focused by a bulk cylindrical lens, after which the light enters the chip through a tailored lens on the right side (Figure 2). The light reflects off the SU-8 mirror and is focused in the center of the channel. The high refractive index of SU-8 (n≈1.6) results in a low critical angle (total internal reflection) when an interface is shared with air. Hence, a SU-8 mirror (sloped SU-8/air interface) allows us to bend the light 90 degrees and perform the measurement and the subsequent light collection orthogonal to the incoming laser beam. A second mirror, on the other side of the channel, ensures that only scattered light reaches the tailored output lens, and, through that, another bulk lens and finally the photo detector. The tailored shape of the on-chip lenses have been optimised by segmenting the front interface into several small line segments and then arranging each of them according to refraction calculations. Figure 3 illustrates the performance of three different lens shapes. In events of Mie scattering (particle diameter ≈ wavelength) most light is scattered in the forward direction. Hence, the challenge when designing a cytometer is to collect scattered light at as small a scattered angle as possible, without collecting the unscattered light.

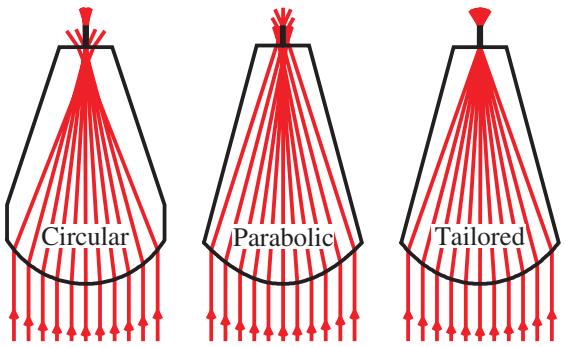

Figure 3: Raytrace simulation showing three differently shaped incoupling lenses. Only the specially tailored lens is able to refract all incoming rays to the 60 µm waveguide (Lenses measure 4 mm x 6 mm).

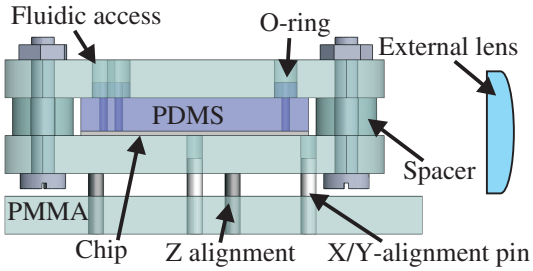

Figure 4: A cross section of the device, showing the layered structure. The chip in this figure consists of patterned SU-8 on a glass substrate. A pattern of pins in the holder is used to insure easy and precise alignment of the chip. Fluidic connections are made with a single custom-made connector, which supports the plug and play concept.

EXPERIMANTAL

The device is fabricated using a single standard UV-photolithography step to pattern an SU-8 layer on a glass substrate. The chip is closed by placing a cast polydimethylsiloxane (PDMS) lid on top of the SU-8 structure. Fluidic in- and outlets are made through the PDMS layer. The glass/SU-8/PDMS chip is placed between two micromilled PMMA parts (cross section shown in Figure 4). These serve to compress the PDMS, thereby keeping the chip sealed, to protect the chip from rough handling and to aid the alignment of the chip when placed in the instrument. The compression is achieved by bolts and prefabricated spacers slightly thinner than the combined chip and PDMS. The X/Y-alignment is achieved by a carefully drilled pattern of 2.1 mm holes in the lower PMMA part and equipping the chip holder platform with a complementary pattern of 2 mm steel pins. The Z-alignment is obtained by letting the lower PMMA part rest on three pins slightly shorter than the aforementioned. The main microfluidic channel is 100 µm wide and 75 µm deep. The on-chip incoupling lens has a focal length of 12 mm. The outcoupling lens focuses the light approximately 200 mm from the chip. The experiments were performed at a constant flow rate of 20 µL/min using a single syringe pump. The tested solutions contained different concentrations of either 1 µm or 2 µm polystyrene particles. For each solution data was collected for 60 seconds at a sampling rate of 80 kHz.

RESULTS AND DISCUSSION

The performance of the cytometer is shown in Figure 5. The two plots show the relation between the number of injected particles and the number of counted particles. It can be seen from the data that when counting 1 µm particles, no more that 6-8000 particles/min should pass. For 2 µm particles it is safe to go up to 12-14000 particles/min. These numbers correspond to concentrations of 3-400 particles/µL and 6-800 particles/µL, respectively. At the current state it is not clear whether these are due to limitations of the chip/setup or the data treatment. In order to obtain a proper particle count, the particle detection threshold must be calibrated for each type of particle.

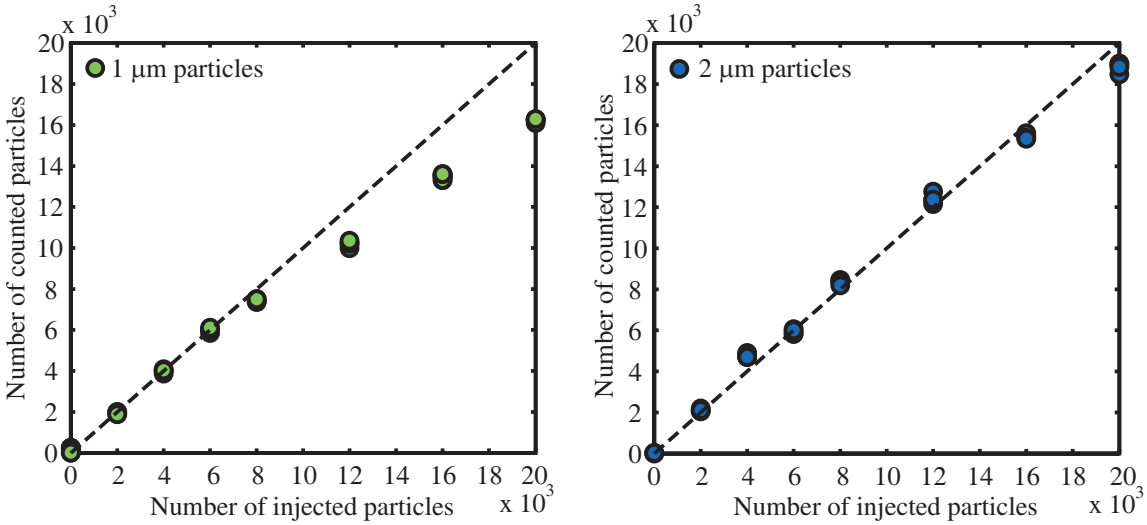

Figure 5: The two plots show the relation between the number of injected particles and the number of counted particles. In both measurements the numbers start to divert from the 1:1 ratio when the number of particles increases. The onset happens much earlier for the 1 µm particles than for the 2 µm particles.

To demonstrate the efficiency of the alignment system a test chip (Figure 6) with four identical but separate pairs of input and output lenses was fabricated and tested. Each lens pair was connected by a 60 µm wide waveguide. Rather than curving the waveguide a sharp 90 degree bend was designed as shown below. The smaller triangle on the inside of the bend was added to prevent the SU-8 from cracking. As shown in Figure 7 the test chip was successfully lifted, rotated by 90 degrees, and replaced 30 times within 2 minutes.

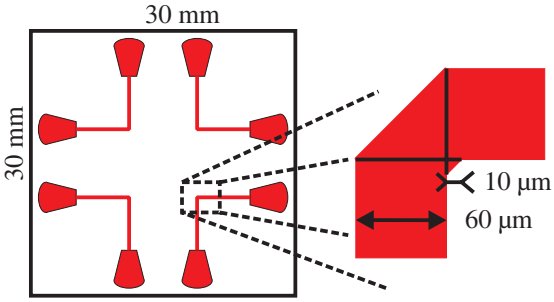

Figure 6: A sketch of the SU-8 test chip used to show the plug and play capability. The shape of the tailored input and output lenses are identical. Each lens is used together with a bulk cylindrical lens to shape the light from a laser beam to fit the chip and vice versa. The high refractive index of SU-8 allows us to use 45° air interfaces as mirrors.

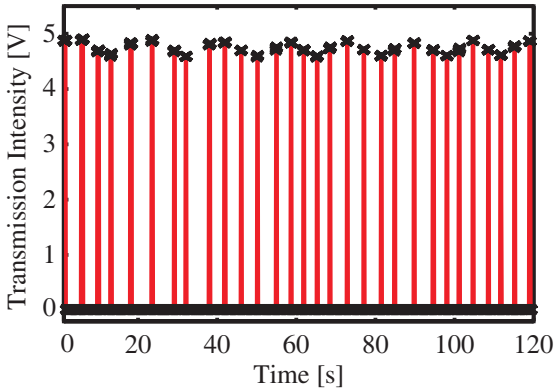

Figure 7: Data showing 30 successful 90° rotations of the test chip performed in 2 minutes. The cyclic pattern is a result of alignment imperfections among the four separate structures.

Though the time it takes to change the chip should not be regarded as a result as such, it should be considered as an indication of the plug and play abilities of the system.

CONCLUSION

We have successfully developed an on-chip cytometer that can either function as a stand-alone device or be implemented as part of a larger LOC-device. We have demonstrated that particles down to 1 µm can be detected and counted reliably at 100 Hz for concentrations below 400 particles/µL. Further more, this device also demonstrates the huge advantages gained from omitting the optical fibers and using a lens based system instead. One advantage is the much easier alignment procedure, leading to a very short chip replacement time. Another advantage is the increased durability and user friendliness of the fiber free chip compared to its fiber based counter parts. It has been demonstrated how the on-chip lenses can be used both for focusing the light directly at the detection channel or to couple light in and out of waveguides. This versatility allows the lens system to be advantageously implemented in many other light based (absorption, fluorescent, etc.) microdevices. Since this cytometer chip does not rely on any flow focusing schemes it is very stable and easy to use. It is, however, dependent on calibration measurements and a clear distinction of differently sized particles in the same solution still needs to be demonstrated.

ACKNOWLEDGEMENTS

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