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From chip-in-a-lab to lab-on-a-chip: a portable Coulter counter using a modular platform

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Abstract

The field of microfluidics has been struggling to obtain widespread market penetration. In order to overcome this struggle, a standardized and modular platform is introduced and applied. By providing easy-to-fabricate modular building blocks which are compatible with mass manufacturing, we decrease the gap from lab-to-fab. These standardized blocks are used in combination with an application-specific fluidic circuit board. On this board, electrical and fluidic connections are demonstrated by implementing an alternating current Coulter counter. This multipurpose building block is reusable in many applications. In this study, it identifies and counts 6 and 11 μm beads. The system is kept in a credit card-sized footprint, as a result of in-house-developed electronics and standardized building blocks. We believe that this easy-to-fabricate, credit card-sized, modular, and standardized prototype brings us closer to clinical and veterinary applications, because it provides an essential stepping stone to fully integrated point-of-care devices.

Introduction

After the initial hype in microfluidics almost three decades ago, which occurred after the introduction of the micro total analysis systems, the field of microfluidics continues to struggle with obtaining widespread market penetration^{1,2}. Lack of standards, focus, and communication between academics and industry could be the reason for this struggle³. Although many lab-on-a-chip devices are presented in the literature, the term chip-in-a-lab fits better with prototypes currently demonstrated in the microfluidic field⁴. Even though there are a few frontier commercialized devices (Abbott i-STAT and DNA PCR machines), most lab-on-a-chip devices are still stuck at a technology readiness level (TRL) of 3 or 4. Figure 1 shows a diagram with different stages of innovation and their corresponding TRLs^{5,6}. If these early prototype devices would become available commercially, they could serve several niche markets and start to

decrease the discrepancy between academic output and actual market revenue.

Currently, most of the microfluidic systems are not compact, due to issues with system integration⁷. Even though auxiliary equipment is part of microfluidic set-ups, it is originally intended for other fields, it is bulky, and not easy to miniaturize⁸. Moreover, components for fluid control, visualization, and signal detection are not interoperable with each other, thus commercialization is prevented^{7,9}. In order to realize true point-of-care (POC) microfluidic devices, this bulky auxiliary equipment should be miniaturized. Decreasing and standardizing the footprint of auxiliary components will be a step in enhancing portability and reducing equipment dependency. Furthermore, it will improve system integration and compactness, ultimately preventing microfluidic systems to end up in the valley of death between academia and industry.

Until now standards have not been established in the microfluidic field, which leads to adoption of equipment from other fields, for example, smart phones for read-out¹⁰, microscope slides, microtiter plates, and Luer

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Fig. 1 Different stages of innovation. Diagram according to Centre for Process Innovation UK and TRLs according to Horizon 2020

connectors¹¹. Therefore, it is difficult to assemble them in one compact system, which often manifest itself as a so-called spider web assembly in the lab where many tubes and wires connect the various parts to each other. Moreover, the microfluidic community prefers a wide variety of substrate materials, including glass, silicon, and polymers, such as polydimethylsiloxane (PDMS). Even though PDMS is a popular material amongst researchers, it lacks high volume and low cost fabrication and does not have long shelf life, making it unsuitable for realizing commercial products^{8,12,13}. In order to commercially benefit from already presented microfluidic devices in academia, which are based on a wide variety of materials, a standardized platform is needed. To provide successful system integration, a large consortium of major industrial and academic partners have introduced standardized elements that maintain compatibility and interoperability between various system parts. Most of the standards that are described in the ISO workshop agreement 23:2016¹⁴ and whitepapers^{15,16} relate to footprint and interconnect positions of smaller microfluidic elements. In this study, microfluidic building blocks (MFBBs) with a standard footprint are prepared according to ISO workshop agreement and assembled on a fluidic circuit board (FCB). The modular platform combines multiple materials, including a glass Coulter counter chip, plastic MFBBs, and an FCB that is fabricated using rapid prototyping. Instead of PDMS, using a polymer like cyclic-olefin copolymer (COC) provides high volume manufacturing while maintaining biocompatibility and Food and Drug Administration (FDA) approval.

In this paper, we reduce the gap between academia and industry one step closer by demonstrating the implementation of a commonly used technology on a standardized platform. The widely applicable alternating

current (AC) Coulter counter is chosen, since it supports label-free detection of single cells, detection of morphological defects and categorization of different cell types¹⁷. The possibility of performing cell sorting, cancer research, and drug screening makes an AC Coulter counter a favorable building block^{18,19}. In this study, the AC Coulter counter previously demonstrated by Segerink et al.²⁰ is combined with already available building blocks²¹. The resulting credit card-sized, modular system provides label-free, real-time detection of different sized beads. Elimination of auxiliary components is not yet possible. However, an in-house developed, portable, and low cost lock-in amplifier based on a digital signal processor (DSP) evaluation board with custom front-end increases the possibility of having it in a MFBB format in the future. The standardized MFBBs facilitate easy transition of this prototype to the market.

Results and discussion

Fabrication results

Owing to utilization of the standard described in ISO Workshop agreement 23:2016¹⁴, individual MFBBs that provide various functions are assembled onto a credit card-sized FCB (Fig. 2a, c(1)). The printed circuit board (PCB) holds all necessary electronics needed for the pseudo-differential measurement and increases the compactness of the system (Fig. 2b). Interfacing and assembling MFBBs (Fig. 2c(2)) onto the FCB (Fig. 2c(1)) provides functionality and interconnection, respectively. Necessary tools for assembly are shown in Fig. 2c(3). These standardized parts can be put in an online repository to be easily integrated in future projects²². The standardization process together with MFBBs facilitates increasing the TRL (Fig. 1) and decreases the gap between the “chip-in-a-lab” concept and a commercial POC device.

Table 1 Number of detected beads in the datasets: video, electrical, and combined

Exp.	Video data No. of beads		Electrical data No. of peaks	Combined No. of video detections matched with electrical peaks					Average bead velocity
	6 μm	11 μm							
				6 μm		11 μm		False positives	
				No.	Detection rate	No.	Detection rate		
6 μm solution	173	0	164	148	86%	0		14	110 μm/s
11 μm solution	84	85	113	27	32%	84	99%	3	344 μm/s
Mixed solution	118	46	101	46	39%	46	100%	9	289 μm/s

Experimental results

The AC Coulter counter chip integrated onto an FCB is used to detect 6 and 11 μm polystyrene beads in phosphate-buffered saline (PBS). Three sets of experiments are conducted. Solutions that contain only 6 μm beads, only 11 μm beads, or both of them in 1:1 (v/v) ratio are pumped through the microfluidic channels. The results for these measurements are shown in Table 1. The video data are used to obtain the trajectory of the bead as well as the size of a bead, which is subsequently linked to the electrical signal. An exemplary result of this process is shown in Fig. 3b. Also, a video showing this process can be found in the Supplementary

Material (Video S1). As Table 1 shows 6 μm beads were detected while running the 11 μm bead solution. Although the system in this case should only detect 11 μm beads as only those kind of beads are present in the bulk solution, some 6 μm beads are still detected from earlier experiments that are left behind in the channels. In order to calculate the detection rate, the number of video detection matched to an electrical signal peak is divided by the total amount of beads detected in the video. The results show that both solutions that contain 11 μm beads have a detection rate of more than 99%. The detection of 6 μm beads was more challenging, since

the peaks generated are close to the noise band (Fig. 3a). One mV_{rms} was deemed to be a suitable threshold for peak detection, since the root mean square (RMS) level of the noise was $0.307 \text{ mV}_{\text{rms}}$. The largest contributor to the noise was the minimal shielding on the FCB. Even though the threshold was set to three times of the RMS level of the noise, some false positives still occurred. The threshold was kept fixed to prevent lowering the detection rate. This threshold resulted in detection rates of 86, 39, and 32% for 6 μm beads at velocities of 110, 289, and 344 $\mu\text{m/s}$, respectively. From these results it is clear that detection rate was negatively influenced by higher flow speeds, since higher flow speeds result in higher frequency components in the detected peaks. Depending on the filter setting in the lock-in amplifier, removal of these high-frequency components leads to a peak amplitude below the threshold for 6 μm beads. Increasing the bandwidth of the filters to allow the higher frequency

components to pass is not favorable as the noise would also increase.

The distribution of detected peak amplitudes for the mixed solution is shown in Fig. 3c. The peak amplitudes for the 11 μm beads are between 1.9 and $20.7 \text{ mV}_{\text{rms}}$, while the majority of peak amplitudes for 6 μm beads is between 1 and $1.8 \text{ mV}_{\text{rms}}$, with an average of $1.6 \text{ mV}_{\text{rms}}$. Since the total amplification of the pseudo-differential measurement electronics is 100 kV/A , it results in a 16 nA current difference between the resistor-capacitor (RC) reference and the microfluidic chip. A 6 μm bead passing the electrodes generates a difference of 0.3% in the total current flowing between the electrodes. The wide distribution of the peak amplitudes for 11 μm beads (Fig. 3c) can be explained by size variation of the beads, as well as their position in the non-uniform electric field between the planar electrodes. The coefficients of variation (CV) supplied by the manufacturer are 18

and 7% for the 11 μm and 6 μm beads, respectively (Fig. 4). This variation results in a small overlap of the populations. Due to this overlap, both populations can generate equal peak amplitudes. Moreover, the peak amplitude of the signal depends on bead volume instead of bead diameter. Therefore, the histogram in Fig. 3c has a broader distribution than the plot in Fig. 4. The two outliers in the 6 μm peak amplitude histogram are 6 μm beads, which were falsely linked to an electrical peak of a 11 μm bead. This occurs when a 6 μm bead is not electrically detected and a 11 μm bead is present in the same 100 ms time interval.

In order to detect particles smaller than 6 μm , a reduction of the noise level is needed. This can be realized by better shielding of the FCB. The presented system could be used for the detection and counting of cells with diameters in the range of 10 to 15 μm like white blood cells. Improvement in the noise level will enable bacteria and yeast cell detection which have sizes in the range of 1 to 6 μm .

Materials and methods

Coulter counter chip

In this study, we combined the Coulter counter chip that is demonstrated by Segerink et al.²⁰ with our standardized platform in order to pave the way for a POC device. This chip consists of coplanar electrodes that are fabricated in a microfluidic channel, as shown in Fig. 5a. An in-house developed lock-in amplifier is used to apply an alternating potential across the electrodes, resulting in an electric field inside the fluidic channel. The impedance of the system increases approximately 0.01%, when an insulating polystyrene bead passes through this electric field.

Design of the microfluidic system

A design philosophy was used where standardization plays a major role. The system consists of standardized MFBBs that provide the functionality of the system along with aforementioned Coulter counter chip. An FCB is designed to connect these functional blocks. Interconnect positions between the MFBBs and the FCB are

circuit package. For bonding, the surfaces of two COC plates are exposed to cyclohexane vapor for 3 min and afterwards immediately pressed together for 15 min at 2.1 MPa pressure at 110 °C (Carver 3889CEB.4NE1001). Mounting holes are drilled, in order to allow anchoring of MFBBs to the FCB using clamps. The grooves for the electrical traces are filled with silver/silver chloride paste (Gwent C2051014P10) to provide electrical connections. Before the paste cures, electrical components are placed, eliminating the need to solder afterwards.

O-rings between the MFBBs and FCB facilitate a fluidic seal. Pogo pins (Smiths Connectors, 101582) are used to obtain an electrical connection between the FCB and the Coulter counter chip. A trans-impedance amplifier is mounted on the FCB and additional electronic circuitry is provided by a separate PCB, which is connected to the FCB with a card edge connector.

Experimental

Six micrometer bead solution (Polystyrene Red Dyed Microsphere, Polyscience Europe, Germany) and 11 μm bead solution (Copolymer Microspheres 7510A, Thermo Fisher, USA) with a concentration of 10^6 beads/mL in PBS (Sigma Aldrich, The Netherlands) are prepared. Tween-20 (Sigma Aldrich, The Netherlands) is added to both solutions with a volume fraction of 0.1% to prevent clustering of the beads. These solutions are thoroughly mixed before they are loaded into the inlet reservoir MFBB. In addition to a height difference of 1 cm between inlet and outlet reservoir, a pressure pump is initially used to introduce fluid into the channel. The current through the Coulter counter chip is recorded at 0.3 V_{RMS} , 90 kHz in real time using a LabView program interfacing the lock-in amplifier. The total amplification of the electronics is 100 kV/A.

The recorded data are subsequently processed with a MATLAB (MathWorks, USA) script, in order to detect peaks that represent beads. A moving average filter is applied to the signal and the result of this operation is subtracted from the original signal to remove drift in the signal. Peak amplitudes and corresponding times are obtained from this processed signal and compared to the video data.

Video data are obtained during the experiment, using a microscope (Leica DM LM and SUSS MicroTec, Germany) and a high speed camera at 60 fps (Grasshopper 3, Point Grey, USA). A MATLAB script is used to analyze the resulting video. The script detects the amount of pixels the bead occupies, in order to identify different bead sizes. The location and size information for each bead is saved to determine the exact time that the bead passes the electrodes. Subsequently, this time is used to find the corresponding peak in the impedance data. Overall this script provides information about detected bead size, peak amplitude, and bead velocity for each bead.

Conclusion

In this paper, we demonstrate a low-cost, modular particle counting microfluidic system with a credit card-sized footprint. The reusable standardized parts decrease the design and fabrication time, which leads to a reduction in the time-to-market for industrial development. The standard platform provides the possibility of integrating different materials in one system. Here, a COC, a PMMA, and a glass device are combined. The low cost of in-house built electronics and lock-in amplifier facilitates a route to commercialization while still keeping system performance up to par. We achieve to measure a 0.3% change in the impedance signal using a pseudo-differential measurement. As a result, 6 and 11 μm

polystyrene beads were differentiated. All 11 μm beads were electrically detected, while the detection rates for 6 μm beads were 86, 39 and 32% depending mostly on the flow rate used. Better control over this flow rate will ensure a higher detection rate in the future. Moreover, better shielding of especially the trans-impedance amplifier on the FCB will reduce the noise levels. Most importantly, we show that this standardized platform bridges the gap from “chip-in-a-lab” to “lab-on-a-chip”. The only drawback of the system is the use of an external lock-in amplifier and a pressure regulator. However, we are confident that we can shrink the size of these two components towards obtaining a commercial product.

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Authors' contributions

S.D. helped to design the system and wrote the article. P.K.I. performed the experiments and helped to write the article. T.F. designed and manufactured the FCB. L.I.S. provided the Coulter counter microfluidic building block and helped to write the article. M.O. helped to design the system and write the article.

Conflict of interest

The authors declare that they have no conflict of interest.

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