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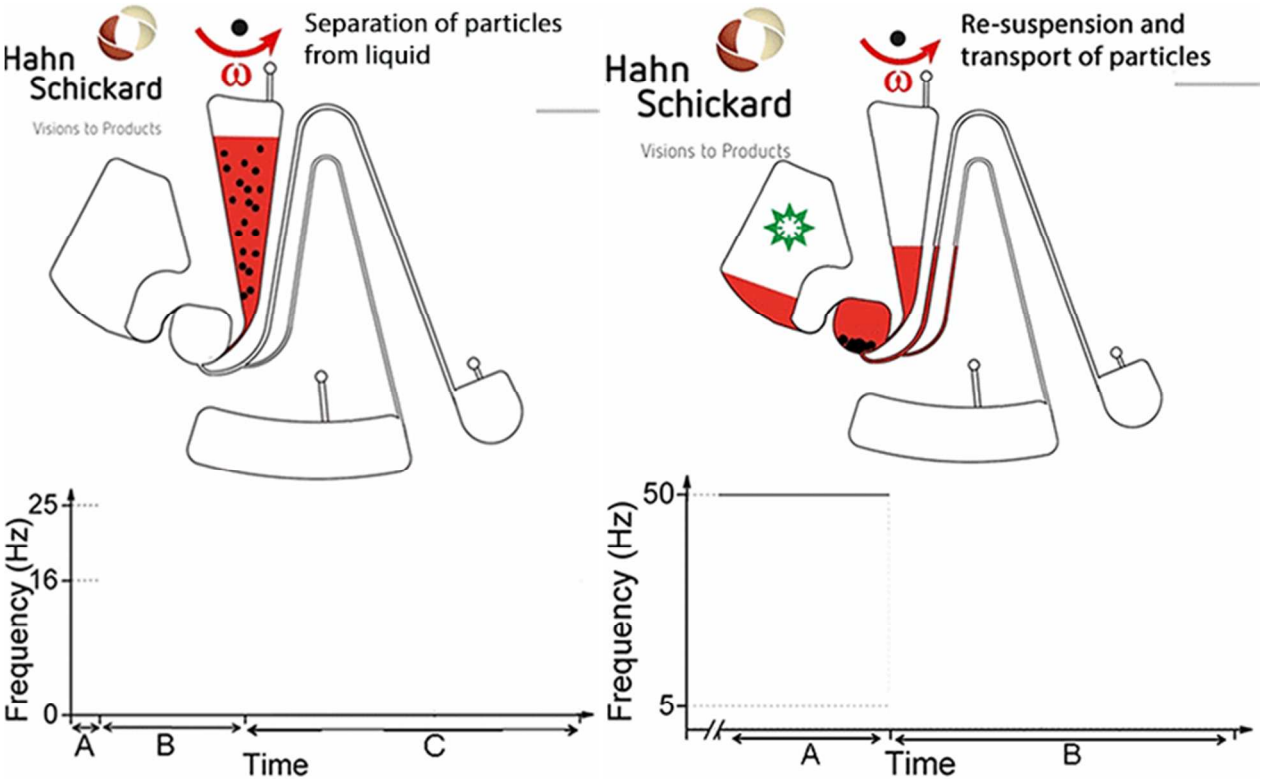

The centrifugo-pneumatic particle sedimentation, re-suspension and transport do not require any auxiliary means besides rotation and can be used for magnetic or non-magnetic particles with different particle sizes and densities.

Journal Name

ARTICLE

Centrifugo-pneumatic sedimentation, re-suspension and transport of microparticles

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Y. Zhao[a], F. Schwemmer[b], S. Zehnle[a], F. von Stetten[a,b], R. Zengerle[a,b,c] and N. Paust[a,b]

Microparticles are widely used as solid phase for affinity based separation. Here, we introduce a new method for automated handling of microparticles in centrifugal microfluidics that is not restricted by the particle size and requires neither auxiliary means such as magnets nor coating of microfluidic structures. All steps are initiated and controlled by the speed of rotation, only. It is based on storage and "on demand" release of pneumatic energy within tunable time frames: A slow release of the pneumatic energy triggers a first fluidic path through which the supernatant above the sedimented particles is removed. An abrupt release triggers a second path which allows for liquid routing and transport of the re-suspended particles. Re-suspension of particles is thereby achieved by quickly changing the speed of rotation. We demonstrate the exchange of the particle carrier medium with supernatant removal efficiency of more than 99.5% and particle loss below 4%. Re-suspension and subsequent transport of suspended particles shows particle loss below 7%. The method targets for the automation of particle based assays e.g. DNA extractions and immunoassays. It is compatible to monolithic integration and suitable for mass production technologies e.g. thermoforming or injection moulding.

Introduction

Separation using microparticles is prevalent for various applications e.g. DNA/RNA extractions and immunoassays.[1,2,3] These assays employ the surfaces of microparticles as solid supports to specifically separate target analytes from a complex mixture. Compared to other techniques, this method offers several advantages. Firstly, various methods are available to functionalize microparticles which facilitates multiplex assays in a single test.[1,4] Secondly, suspension of microparticles within a homogeneous phase results in a short diffusion length between analytes and the surface of particles which enables fast affinity reactions. Moreover, the large surface to volume ratio provides higher binding capacity. Thus, a fast reaction with high sensitivity consuming low volumes of reagents may be achieved within finite incubation times.[5]
Generally, in standard particle based assays, functionalized microparticles get in contact with sample to specifically bind target analytes. Afterwards, washing steps remove all unbound species where complete separation of particles from the sample and re-suspension of particles are essential to achieve high purity and sensitivity.[6] Finally, an elution step (DNA extraction) or direct detection of analytes bound to the particles may be performed. This requires sufficient re-suspension and subsequent separation (from DNA elution) or transport of microparticles (to a detection area). For all unit operations, low particle loss is mandatory to achieve high sensitivity.[6] Hence, an efficient method for handling of microparticles is particularly required.
Among the available microfluidic technologies for handling of microparticles,[3] centrifugal microfluidics provide specific advantages such as simple liquid actuation without interfaces to external pumps,[7,8] bubble free liquid handling enabled by centrifugal buoyancy as well as scalable centrifugal forces for sedimentation.[8,9] Most existing approaches in centrifugal microfluidics employ external magnets to assist handling of microparticles. Using the direct interaction between magnets and magnetic particles, re-suspension[10] and transport[10,11] of microparticles can be achieved. However, these methods are limited to magnetic particles. Moreover, coating of microfluidic structures may be required to support transporting of particles.[10] Another method includes a steel wire in a microfluidic chamber where microparticles are stored. A magnet is employed to agitate the steel wire to re-suspend the particles. Separation of microparticles from the carrier medium is achieved by sedimentation of the particles through a density medium (the medium is denser than the particle carrier medium but less dense than the particles). In this way, microparticles are washed without multiple rinsing steps.[12] This method has strict requirement on the density of the particles and the medium. Instead of employing external means, building physical structures for handling of particles is also prevalent. Particle aggregation by geometric constriction has been demonstrated to be an appropriate method for

[a] Hahn-Schickard, Georges-Koehler-Allee 103, 79110 Freiburg, Germany. Email: yunpeng.zhao@Hahn-Schickard.de
[b] Laboratory for MEMS Applications, Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany.
[c] BIOSS – Centre for Biological Signalling Studies, University of Freiburg, Schänzlestr. 18, 79104 Freiburg, Germany.
Electronic Supplementary Information (ESI) available: See
DOI: 10.1039/x0xx00000x

particle based assays.[13,14,15] Another method employs an array of V-cup barriers to trap particles.[16] For both approaches, the packing density is limited and particles are comparatively large. The resulting small surface to volume ratio and large diffusion length requires long incubation times to achieve sufficient binding of analytes at low concentrations.[2,5]

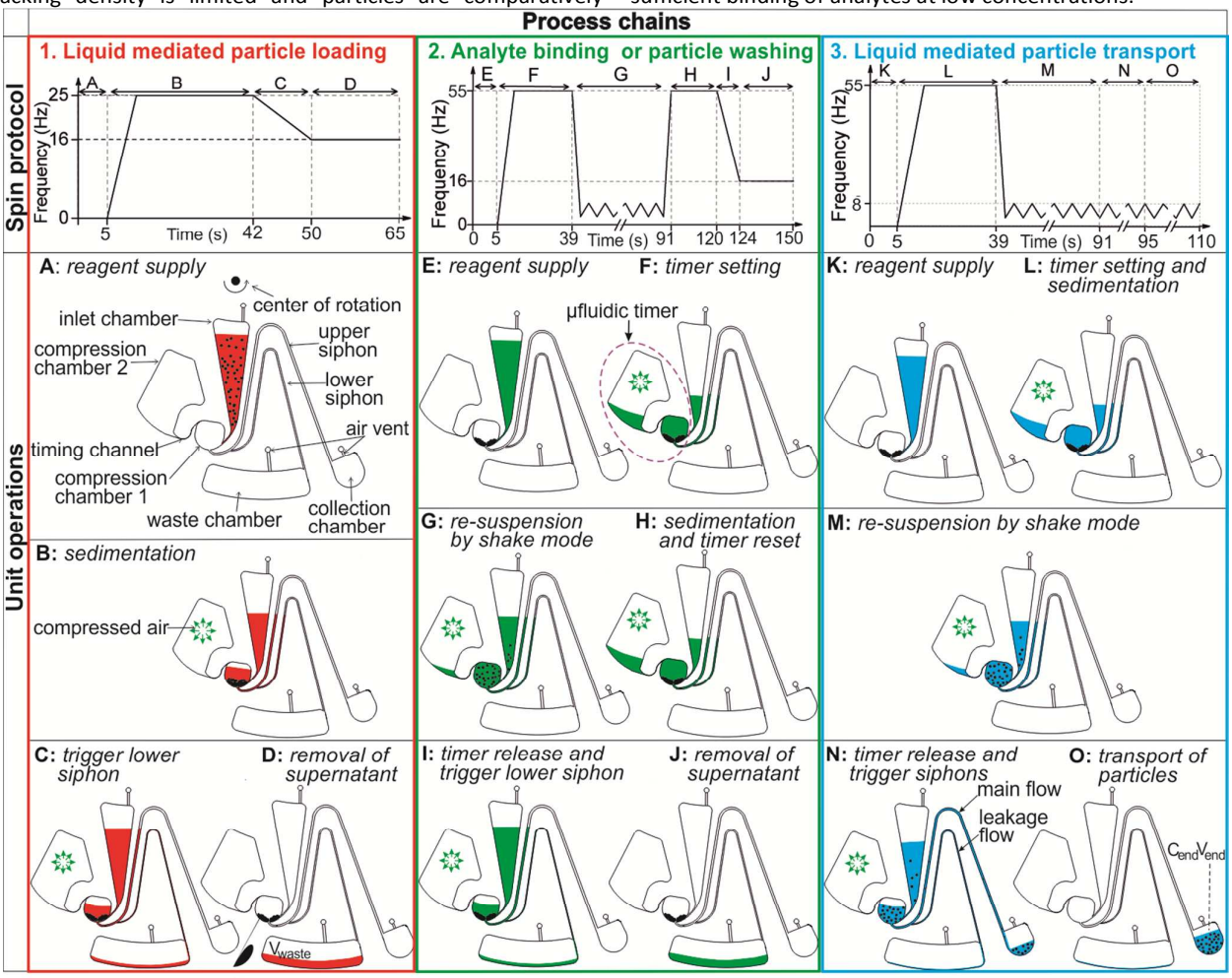

Fig. 1: Schematic illustrations of process chains for centrifugo-pneumatic sedimentation, re-suspension and transport of microparticles and the corresponding spin protocols. **Process chain 1: Liquid mediated particle supply**: A) Loading of particle suspension B) Upon centrifugation, the suspension fills the first compression chamber and compresses air; microparticles sediment. C) Upon slow deceleration to a medium spin frequency, the compressed air is released steadily displacing liquid out of the first compression chamber. The liquid levels in the inlet chamber and the siphons are elevated equally until the lower siphon with a high fluidic resistance is triggered. D) Supernatant is removed into the waste chamber while particles remain sedimented in the first compression chamber. **Process chain 2: Analyte binding and particle washing:** E) Load sample or wash buffer. F) Above a critical frequency, the first compression chamber is overfilled priming the timing channel and liquid fills the second compression chamber to set the timer. Microparticles sediment in the first compression chamber. G) Upon deceleration, siphon priming is delayed due to the high fluidic resistance in the timing channel. Re-suspension of microparticles is achieved by shake mode during the delay period. H) Particles are re-sedimented before the second compression chamber is empty. I) A slow deceleration to a medium frequency enables priming of the lower siphon. J) The supernatant is removed into the waste chamber. **Process chain 3: Liquid mediated particle transport** K) Loading of another liquid L) sedimentation and setting of the timer. M) Re-suspension by shake mode until the second compression chamber is empty. N) The pneumatic energy is released abruptly priming both siphons. O) Re-suspended microparticles are transported together with the liquid through the upper siphon with low fluidic resistance to the collection chamber.

We present a centrifugo-pneumatic method for microparticle re-suspension, transport, and sedimentation with subsequent separation of the particles from the carrier liquid. It is neither restricted to the size or magnetic properties of particles nor requires any coating of microfluidic structures. All steps are automated by programming the rotation speed exclusively. The method employs the recently presented microfluidic timer.[17,18] The timer is used for time controlled release of previously stored pneumatic energy and is connected to two siphons with their crests at two different radial locations (Fig. 1-A). The spin protocol is used to either quickly or slowly release pneumatic energy which enables to trigger either of the two siphons. Based on the two main features, the time controlled release of pneumatic energy and two siphons for liquid routing, the functional principle of the particle handling can be categorized into three microfluidic process chains [9]: 1) liquid mediated particle loading, 2) analyte binding or particle washing and 3) liquid mediated particle transport. Each process chain in turn consists of a series of unit operations that describe the workflow as illustrated in Fig. 1. Dimensions of the microfluidic structure are detailed in the ESI.

Liquid mediated particle loading (Fig.1-process chain 1): Upon centrifugation, particles along with their carrier liquid medium are transferred into the compression chambers. During this operation, the enclosed air in these chambers is compressed while the microparticles are sedimented to the bottom of the first compression chamber. The radial positions of the liquid menisci within the inlet chamber and the siphons are defined by two forces balancing each other: i) the centrifugal force at 25 Hz, directed radially outwards, and ii) the pneumatic force from compressed air, pushing liquid radially inwards (Fig. 1-B). A slow reduction of spinning frequency to 16 Hz defines a new equilibrium position for the liquid menisci located radially in-between the crests of the two siphons. In this way the lower siphon is primed exclusively. As a consequence, the supernatant is sent to the waste chamber, while the particles remain sedimented in the first compression chamber.

Analyte binding or particle washing (Fig. 1-process chain 2) both require sufficient re-suspension of microparticles and afterwards complete separation from the liquid. Re-suspension of microparticles is achieved by periodically changing the rotation speed with acceleration/deceleration rate of 67 Hz/s between two low frequency limits. This concept has been demonstrated previously and is referred to as shake-mode.[19] During this operation, the microfluidic timer is used to prevent any siphon priming at such low rotation speed. Consequently, the maximum duration for shake mode re-suspension is defined by the timer delay period. If a longer re-suspension time is required, the timer must be reloaded by temporarily increasing the spinning frequency. Mixing could be further enhanced by bidirectional shake mode (see videos in ESI). Separation of the liquid is achieved by sedimentation and subsequent removal of the supernatant through the lower siphon as described in the process chain #1.

Liquid mediated microparticle transport enables transport of microparticles together with an aqueous carrier medium (Fig. 1-process chain 3). The timer is loaded and the microparticles are re-suspended within the carrier medium by shake mode at low spinning frequencies. The shake mode continues until the second compression chamber is empty. The pneumatic energy is then released abruptly and both siphons are triggered at frequencies below 10 Hz. Due to the high fluidic resistance in the lower siphon, only a minor amount of the particle suspension is lost with the leakage flow through the lower siphon. Almost all re-suspended particles are transported together with the liquid through the upper siphon to the collection chamber.

Materials and methods

The microfluidic structure was designed in Solidworks (Dassault Systèmes S.A., France) and manufactured by Hahn-Schickard Lab-on-a-Chip Design & Foundry Service (Freiburg, Germany). In brief, the structure was milled on a 130 mm diameter and 6 mm thick PMMA disk using a precise micro milling machine (KERN Microtechnik GmbH, Germany). Afterwards, the disk was cleaned with isopropanol, rinsed with DI water, dried with nitrogen gas and sealed with polyolefin adhesive foil (900360, HJ-BIOANALYTIK GmbH, Germany). Microparticle suspension (Dynabeads® M-280 tosylactivated with diameters of 2.8µm and a density of 1.4g/cm^3) and phosphate buffered saline (PBS) were purchased from Life Technologies AS, Norway. The bovine serum albumin (BSA) was purchased from Carl Roth GmbH, Germany. The particle suspension was diluted in 0.1 M PBS containing 0.1% (w/v) BSA. For each experiment, 150 µl (V_{ini}) of the diluted particle suspension was loaded into the disk. The disk was processed in a stroboscopic setup according to the spin protocols in Fig. 1. The stroboscopic setup synchronized with an optical setup enables recording of real-time images during disk rotation. All experiments and measurements were performed in triplicates.

Quantification of particle loss was realized by an in-house developed particle concentration measurement method (see ESI). The concentration of the particle suspension was determined by measuring the autofluorescence signal (Fig. 2). To quantify the particle loss during removal of supernatant (PL_s), the microparticles sedimented in the first compression chamber (Fig. 1-D) were manually re-suspended by pipetting with 150 µl (V_{res}) PBS containing 0.1% (w/v) BSA and the particle loss was determined by comparing the initial particle concentration c_{ini} to the resuspened concentration c_{res}:

$$PL_s = \left(1 - \frac{c_{\mathrm{res}} \cdot V_{\mathrm{res}}}{c_{\mathrm{ini}} \cdot V_{\mathrm{ini}}}\right)$$

where V_{ini} and V_{res} is the volume of the initial and resuspended particle suspension, respectively. Similarly, the particle loss during transport (PL_t) was:

$$PL_{\mathrm{t}} = \left(1 - \frac{c_{\mathrm{end}} \cdot V_{\mathrm{end}}}{c_{\mathrm{ini}} \cdot V_{\mathrm{ini}}}\right)$$

where c_{end} and V_{end} are the concentration and volume of the particle suspension in the detection chamber after transport, respectively (Fig. 1-O).

The supernatant removal efficiency is defined as the volume ratio between liquid removed to the waste chamber (V_{waste}) and the volume of initial liquid in Fig.1-J. It was determined by measuring the volume of residual liquid (V_{res}) in the compression chambers and siphons after removal of supernatant (details in ESI). Consequently, the supernatant removal efficiency (E_s) was determined by:

$$E_{\mathrm{s}} = \frac{V_{\mathrm{waste}}}{V_{\mathrm{ini}}} = \left(\frac{V_{\mathrm{ini}} - V_{\mathrm{res}}}{V_{\mathrm{ini}}}\right)$$

Results and discussions

Table. 1 Particle loss during removal of supernatant (n=3)

c_{ini} (µg/ml)	V_{ini} (µl)	c_{res} (µg/ml)	V_{res} (µl)	PL_s(%)
163±1	150	160±1	150	2±1
339±8	150	333±2	150	2±2
432±6	150	425±5	150	2±2
921±13	150	911±4	150	1±1

Particle loss during removal of supernatant: Results of the quantification of particle concentration and particle loss are summarized in Fig. 2 and Table 1, respectively. It can be seen that the particle loss during removal of supernatant is below 4 % for four initial particle concentrations. For typical assays with one incubation step and two washing steps, for the

considered particle concentrations, the total expected particle loss would be 7 % ± 2 %.

Re-suspension efficiency and particle loss during transport was evaluated for four different initial concentrations as well (Table. 2). The particle loss after transport was demonstrated to be 7 % or less in all cases. It was mainly due to a minor amount of microparticles that were not successfully re-suspended during shake mode. They remained sedimented in the first compression chamber. In addition, a minor amount of microparticles are transported to the waste chamber due to the leakage flow through the lower siphon. Since transport of microparticles was performed after re-suspension of microparticles, the 7 % or less particle loss during transport (Table. 2) implied that at least 93 % of the sedimented microparticles were successfully re-suspended.

Table. 2 Particle loss during transport (n=3)

c_{ini} (µg/ml)	V_{ini} (µl)	c_{end} (µg/ml)	V_{end} (µl)	PL_t (%)
163±1	150	161±1	146.8±0.1	3±1
339±8	150	333±2	147.5±0.2	4±1
433±6	150	414±5	147.2±0.2	6±1
921±13	150	918±4	147.4±0.2	2±2

The time required for timer setting (Fig. 1-F) and the delay time for re-suspension of the particles (Fig. 1-G) depend on the viscosity of the liquid carrier medium and the dimension of the microfluidic channels.[18] For the specific configuration within this work (viscosity: 1.09 mPa·s and channel dimension as in ESI), when the timer is set at 55 Hz for 30 s, the delay time for re-suspension of microparticles can be sustained for 44 s ± 1 s.

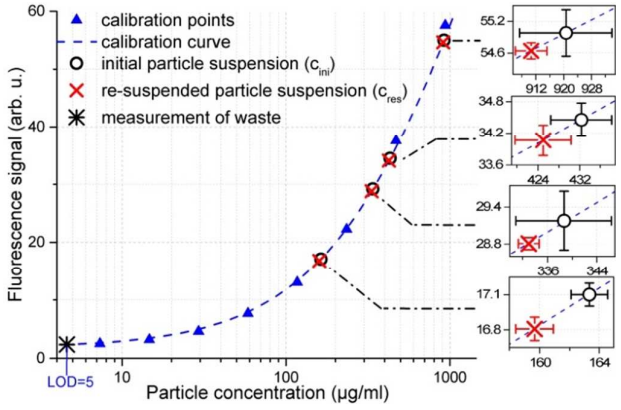

Fig. 2: Autofluorescence signal of the particle suspension versus particle concentration. Calibration points are obtained by measuring a series of particle suspensions with known concentrations. The calibration curve is obtained by curve fitting to the calibration points. By measuring a fluorescence signal, the corresponding particle concentration can be calculated from the fitting curve.

Table. 3 Supernatant removal efficiency (n=3)

c_{ini} (µg/ml)	V_{ini} (µl)	$V_{residual}$ (µl)	E_s(%)
163±1	150	0.7	>99.5
339±8	150	0.7	>99.5
433±6	150	0.6	>99.5
921±13	150	0.7	>99.5

The supernatant removal efficiency: The volume of residual liquid is independent of the initial particle concentration and is in all cases below 0.7 µl (Table. 3). Consequently, the supernatant removal efficiency is in all cases above 99.5 %.

Conclusions

A new method for handling of microparticles in centrifugal microfluidics that does not require any additional external actuation besides centrifugation was introduced. It can be integrated monolithically and is compatible to scalable fabrication technologies such as injection moulding or thermoforming. Within three microfluidic process chains, we successfully demonstrated re-suspension of microparticles, separation of microparticles with liquid carrier medium and transport of microparticles. The new particle manipulation technique can be used in microfluidic automation of affinity based separation within immunoassays, DNA purification or the separation of rare cells and liposomes.

Acknowledgements

We gratefully acknowledge the funding by the federal ministry for education and research (EasyTube, FKZ 16SV5455).

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