Perpetual sedimentation for the continuous delivery of particulate suspensions

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Particles in a rotating fluid experience gravity-directed orbits to remain in suspension.

**Abstract**

Particle sedimentation is deleterious to a tremendous variety of microfluidic applications. Using an open instrumentation approach we show that syringe rotation retains particles in a suspended state, providing a universal solution for the continuous delivery of particulate samples to microfluidic processors.

**Introduction**

Microfluidics has gained maturity to enter many fields and industrial applications with the promise of precision sample processing and quantitative analysis. Microfluidics is increasingly being harnessed for processing particulates in the form of samples, reporter reagents or components for assembly. Applications continue to expand, including biological research1, personalised medicine2, environmental monitoring3, synthetic chemistry4 and artificial life5. However, particles are seldom neutrally buoyant. Sedimentation (or buoyancy) scales with the square of the particle diameter (*D2*) and density dissimilarity (*p–f*) between the particle and the fluid as described by Stokes’ law6 (*U=((p-f)gD2)/18*, where *U* is the sedimentation velocity, *g* is acceleration due to gravity and ** the dynamic viscosity of the fluid). This arises from the general equation for particle motion in a fluid, under the assumption of a quiescent fluid and zero particle acceleration, and equates the particle drag force with the gravity/buoyancy force. Therefore, this generalised problem especially impacts the handling of large particles such as >100-m-diameter human oocytes for IVF7 or transcriptome capture beads8, and dense particles such as magnetic capture beads (**=1.4*–*2.5 g/cm3)9. In the delivery reservoir (*e.g.* syringe) upstream of the microfluidic processor the volume is often effectively stagnant. Here sedimentation effects can become substantial. This poses the risk of sampling bias with heterogeneous samples such as blood or environmental pollutants. In addition, high throughput is a major driver for many contemporary microfluidic applications. Here, sedimentation manifests during prolonged processing required to isolate rare particles, such as circulating tumour cells (1 in 109 haematological cells)10, impacting isolation yield. Sedimentation also effects applications that require tight control of suspension concentrations to create ordered arrangements for efficient cell encapsulation in droplets11-13 or for the efficient microfluidic manufacture of composite assemblies14.

Despite wide-ranging impact, sedimentation is often overlooked, or conventionally managed by manual agitation or preferably by automated magnetic stirring. Magnetic stirring does, however, come with drawbacks; (i) dispersing particles to create inhomogeneous distributions, (ii) introducing shear forces that damage delicate particles such as stem cells and protein crystals; (iii) is impractical for small syringe volumes (relevant to fine needle aspirate biopsies); and (iv) aggregates magnetic particles.

In pursuit of a universal solution we consider the use of syringe rotation such that the particles continuously fall through rotating fluid layers, with each particle describing a gravity-driven miniature orbit superimposed on a macroscopic orbit described by fluid rotation. In this approach, all particles are acted upon, the suspension remains homogeneous and, moreover, the method is suitable for handling even the most delicate particles. We have called the concept perpetual sedimentation (PS) and reduced it to practice using an open instrumentation (OI) approach15-19. Here the merits of OI is not just cost effective performance and a route for technology dissemination but also the ability to introduce functionality that is not commercially available. In this contribution, we describe the performance of a PS prototype, observe the underlying particle transport phenomena and demonstrate fit-for-purpose application with large particles, magnetic particles and cells.

**Materials and Methods**

A detailed account of the materials and methods can be found in the Supplementary Information. In brief, laser cutting and widely available electronics and other components were used to assemble the PS prototype (see SI Fig 1 and 2, and the Perpetual-Sedimentation repository20 for design files, instructions and operating code). To prevent cable and tubing entanglement the pumps rotate 360° and then reverse direction. A rotating microscope was also assembled to investigate particle behaviour within a rotating syringe mounted on the same axle. Microfluidic experiments used 5 mL syringes (12.07 mm ID). To measure particulate delivery a 95-mm-long Dean entrainment microchannel was used for periodic particle spacing and fabricated by soft lithography. This also demonstrates an application scenario requiring high-density particulate flows. A high-speed camera (Miro Lab310, Vision Research) was used to record entrainment and a bespoke intensity profile MATLAB code was used for automated image analysis.

**Results and Discussion**

The perpetual sedimentation concept is illustrated in Figure 1(A) and was demonstrated using an OI approach. This involved laser cutting 5-mm-thick PMMA sheets to provide a rapid means for the digital fabrication of sturdy parts, and once assembled orthogonal to the steel rod framework provides a robust assembly. In addition, the choice of off-the-shelf electronics allows inexpensive and reliable system control.

The OI syringe pumps were characterised; the precision lead screw coupled with microstepping (1/16) enable the reliable and accurate delivery of desired flow rates (tested in the L/min range) with performance equivalent to commercial pumps (see SI Fig. 3). For both open and commercial syringe pumps, stepper motor noise becomes increasingly evident at lower flow rates. However, in practice, dampening by the elastic polythene tubing and PDMS materials enables the scalable kHz generation of droplets in the nanolitre to femtolitre range (see SI Fig 3). The pumps are also sufficiently powerful to manage a back pressure of 1,300 kPa when driving m/s flows of viscous fluid (50% (v/v) PEG3550), whereas the commercial pump was self-limited by flow rate to 950 kPa. At pressures above 1,300 kPa the interconnect failed.



**Figure 1.** The PS concept illustrated with orbit sequences for 10- (red), 20- (blue) and 30-m-diameter (green) particles within the cross section of a syringe (grey) (A). The PS pump prototype with key components identified. Motion is indicated using blue lines; linear displacement by rotation of the lead screw through the locking bushing nut for fluid delivery, and driving gear wheel rotation (anti-clockwise) to rotate the syringe pump gear wheel (clockwise) (B). The driving gear wheel from the chassis is illustrated, but other components (*e.g.* ball bearings) are omitted for simplicity.

A PS system comprising three independent pumps was developed for demanding Drop-seq experiments requiring two rotating syringe pumps for the gentle and continuous delivery of transcriptome reporter beads and cells, and a third static syringe pump for delivery of the carrier oil (see SI Fig. 1 and 2). Illustrated in Figure 1(B) the pumps have a central gear wheel that couples to a stepper motor to achieve rotation. The powerful NEMA17 motor (54 N.cm holding torque; 1/8 microstepping) readily manages the rotation of two syringe pumps at 0.05–0.5 Hz (3–30 rpm; see SI MOV1 for rotation of the lead screw for syringe displacement (200 L/min) and 0.2 Hz rotation of syringe pumps themselves). Stepper motor noise and vibration become evident at lower and higher frequencies, respectively. The complete assembly with 3 independent pumps is inexpensive (~£400), lightweight (4.45 kg) and compact (333x277x155 mm; *l* x *d* x *h*), suitable for inclusion in the vast majority of microfluidics applications. Equivalent performance typically requires three single commercial pumps (*e.g.* Chemyx 100), each costing ~£1,500, weighing 2.94 kg and being 240x170x110 mm in size.

The PS concept considers that syringe rotation produces rotating fluid films and during a revolution the particulates continuously sediment relative to the fluid. As a result the particles experience a millimetre-scale rotation path superimposed with a gravity-defined orbit. This approach acts on all particles within the syringe, is gentle and indiscriminate of the nature of the particle. To gain a better understanding of the particle transport phenomena, an on-board rotating microscope was assembled (see SI Fig. 4). The liquid is viscously coupled to the syringe wall, initially lagging the wall velocity before attaining steady state radial velocity by 3 rotations after which the effects of sedimentation can be accurately measured. As expected, the orbital radius of 30-m-diameter particles decreases with rotation frequency, from 218 m at 0.1 Hz (6 rpm) to 55 m at 0.5 Hz (30 rpm) (Figure 2A and 2B). These orbit scales indicate the 0.1 Hz–0.5 Hz rotation frequencies are adequate for the 12.07 mm ID syringe. Lower frequencies allow further contact with the syringe wall, risking particle depletion. Considering the net displacement velocity, a particle falls 79 m/s due to gravity alone whereas all rotation frequencies tested produce net displacement velocities close to zero (Figure 2C). In practice, rotation is reversed every 360° to avoid tubing entanglement. As shown in SI Figure 5, acceleration and deceleration prevent the fluid from matching the syringe radial velocity to hinder the accurate measurement of gravity directed orbits. Either way, PS allows particles to remain suspended throughout microfluidic experiments allowing, for example, continuous Dean entrainment for the efficient co-encapsulation of single cells with transcriptome reporter beads.



**Figure 2.** Brightfield images of 30-m-diameter particle orbits processed using ImageJ (see SI). A heat map was superimposed on single orbits to confirm the transport path for 0.1–0.5 Hz rotation frequencies and compared with sedimentation without rotation (A). The orbit radius decreases with rotation frequency (mean and standard deviation of 10–21 particles with 46–100 data points per orbit (green)) with close agreement to theory (blue) (B). The net particle displacement velocity resulting from sedimentation alone (red) is compared with displacements during rotation (mean and standard deviation from 10–21 particles for each condition (C)).

In this study we have used polystyrene and magnetic particles along with mammalian cells to evaluate the benefits of perpetual sedimentation for the continuous delivery of particulate suspensions to microfluidic processors. To inform suitability for other common microfluidic applications involving a range of particle scales and density dissimilarities we have provided a sedimentation/buoyancy velocity list (SI Table). To estimate suitable rotation frequencies required to limit wall contact (risk depletion) the scale of the particle orbits can be calculated using a first principle approximation: The fundamental equation for the acceleration of a spherical particle in a continuum fluid is provided by Maxey and Riley21, and under the conditions noted above defines the terminal velocity of a sphere in a quiescent flow. If the density ratio between the particle and the fluid is several orders, then the dominant forces are drag and gravity. If the particle momentum relaxation time (**=*pD2*/18**) is small, relative to the timescale of the applied acceleration, then the particle will approach terminal velocity. For the results shown in Figure 2A, a 30-m-diameter polystyrene particle with *pf* ≈ 1.1 gives ** of the order of 10 microseconds. This is considerably shorter than the time taken for one revolution of the syringe. The terminal velocity equation noted above can be recast as *U = g* and therefore the radius of the particle orbits shown in Figure 2 is *Ro = gT/4* where *T* is the time period for one revolution of the syringe. As shown in Figure 2B, this simple relation gives a good estimate of the relative particle motion in the syringe to guide system design. At higher rotation rates, orbits become elliptical, indicating that other forces become relevant. As to how these forces impact the maintenance of particle suspensions requires further investigation.

To evaluate the feasibility of PS for the continuous delivery of particulate suspensions, a challenging sample containing 30-m-diameter polystyrene particles was tested and compared with magnetic stirring in both horizontal and vertical syringe orientations. A powerful, albeit costly, magnetic stirrer (Multi Stirrus™) was used to allow gentle actuation of the stirring element. Dean entrainment (*Ūflow*=100 mm/s (*κ*≈2.8)) was used as a means to periodically space particles for automated image analysis using a profile intensity method (see SI) that generates a particle delivery barcode (see Figure 3A). Entrainment is established in ~1 minute and during a ten-minute time course the effects of gravity (*i.e.* sedimentation without rotation) in a horizontal syringe results in rapid particle depletion, with zero particles delivered after 6 minutes (see Figure 3B). Magnetic stirring in a horizontal syringe has little effect, with only residual numbers (~5%) of particles delivered after 6 minutes. Here, the actuation of a disc magnet acts to disperse the particles from the actuated fluid volume and, once distant, the particles rapidly sediment (see Figure 3C). In stark contrast, vertical syringe orientation allows the sustained delivery of particles at starting concentrations (see Figure 3D). Here the disc magnet disperses particles downwards to exit the syringe, and in other cases to the nearby syringe wall and upwards to return by sedimentation. Both instances allow repeated dispersion before downwards delivery to the microfluidic circuit. The PS approach also matches this performance enabling the continuous delivery throughout the 10-minute time course with no evident frequency dependence when comparing 0.1 Hz (Figure 3E) with 0.33 Hz (Figure 3F) rotational frequencies.



**Figure 3.** Comparison of in-syringe actuation methods for the continuous delivery of 30 m polystyrene particles to a Dean entrainment microfluidic element. Entrainment and particle count time course examples (A). Delivery control by sedimentation (red) in a horizontal syringe (B); delivery by magnetic actuation (blue) from a horizontal (C) and a vertical (D) syringe; delivery by PS (green) at 0.10 Hz (E) and 0.33 Hz (F). The delivery rate (counts/s) was normalised to account for dissimilar starting concentrations (~0.4M/mL; ~500/s). Mean and standard deviation of 3 experiments are plotted.

We further evaluated the performance of PS in scenarios less suited for magnetic stirring. Magnetic particles common to many microfluidic cell and molecular biology applications9 aggregate in magnetic fields. This creates an inhomogeneous suspension and depletes the effective concentration, making it an entirely inappropriate solution for continuous microfluidic delivery. Even small (8.2 m) and light polystyrene particles doped with magnetic nanoparticles rapidly sediment, with delivery rates <50% after 15 minutes, whereas PS provides continuous particle delivery (Figure 4A).



**Figure 4.** Magnetic particle delivery time course resulting from sedimentation (red) and PS (green) actuation methods (A). THP-1 monocytes delivery time course resulting from sedimentation (red), horizontal magnetic stirring (blue) (B), and PS (C) actuation methods. The delivery rate (counts/s) was normalised to account for dissimilar starting concentrations (~1M/mL; ~250/s). Mean and standard deviation from three experiments are plotted. ATDC5 cell viability (D) following 10 minute storage in a syringe; control (white), with PS (green) and with magnetic stirring (blue). Mean and standard deviation from six samples are plotted. ChemGene bead fragmentation resulting from magnetic stirring (left) compared with beads managed with PS (right) (E).

Importantly, the PS approach is a non-contact method. Moving to a typical mammalian cell, THP-1 monocytes (13.9±1.8 m), a similar sedimentation profile was observed (Figure 4B). Both magnetic stirring (Figure 4B) and PS (Figure 4C) retain cells in suspension throughout the 30 minute experiment (here, the cells are sufficiently light to continuously experience convective transport in a horizontal syringe). However, even hardy cell lines (ADTC5) are affected by the shear conditions imposed by magnetic stirring. Following a 10 minute treatment, cell viability was reduced (*p*<0.0001) to ~90% relative to controls, whereas viability levels following PS treatment matched (*p*=0.7) storage in a stationary syringe at ~95% (Figure 4D). It is worth noting that cell death is an overt end-point, overlooking other cell compromising effects or behavioural alterations. We anticipate PS will be especially suited to more delicate primary cells and especially cells such as neutrophils that are prone to activation. In other scenarios fragile particulates are processed: The costly and brittle ChemGene (ToyoPearl® resin) beads used for Drop-seq single cell transcriptomics are prone to fracture during prolonged (≥20 minutes) magnetic actuation (see Fig. 4E). In contrast, bead damage was not observed during equivalent PS experiments.

In summary, digital fabrication and consumer electronics can be combined to produce high-performance instruments with new functionality. The OI approach allows inexpensive and straightforward dissemination, and favours community-driven iteration and reconfiguration for different microfluidic applications. The PS approach involves radial transport by fluid rotation coupled with orbits created by gravity to retain all particles in suspension. This provides a universal, highly efficient, yet gentle, means for the continuous delivery of particle suspensions to microfluidic processors. Critically this allows the benefits of microfluidics to be reliably translated from research scenarios to real-world industrial applications.

**Conclusions**

Perpetual sedimentation represents a straightforward and universal solution for retaining particles in a suspended state for continuous delivery to microfluidic systems to enable high throughput, unbiased processing without damage. The application space is vast, allowing improved analytical and synthetic performance across many fields.

**Author Contributions**

JW conceived the idea and wrote the manuscript. SL, JB, JH, JS and JW undertook the investigation. TU and JW provided supervision and SL, JB, JH and JS reviewed the manuscript.

**Conflicts of Interest**

There are no conflicts of interest to declare.

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