Rapid on-chip multi-step (bio)chemical procedures in continuous flow – manoeuvring particles through co-laminar reagent streams

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We introduce a novel and extremely versatile microfluidic platform in which tedious multi-step biochemical processes can be performed in continuous flow within a fraction of the time required for conventional methods.

Tedious multi-step reaction processes are a common feature of many (bio)chemical procedures. A good example of this is oligonucleotide synthesis, where the addition of just a single base to a strand of DNA involves four reaction steps and four washing steps.[1] This form of synthesis is extremely important for the production of DNA primers and probes and it is of great significance to researchers interested in artificial genome production.[2] Other examples include bioassays, drug screening, chemical synthesis and catalyst screening.[3] Despite the implementation of large scale automation, these procedures often involve highly repetitive processes that remain time consuming, labour intensive and wasteful of expensive reagents.

Microfluidics is revolutionising fluid handling in analytical chemistry and (bio)chemical synthesis.[4] In micro devices, fluid flow behaviour is predictable and easy to control and, since diffusion distances are small, chemical processes can be performed far more quickly than with macroscale systems. Chemical analysis can be performed with reduced amounts of sample and reagents. Mass transfer can be further enhanced by the utilisation of surface functionalised particles[5] inside microfluidic channels.[6–8] Magnetic microparticles can be manipulated elegantly by external magnetic forces and many such particles are now commercially available with a range of different surface chemistries.[9] A typical example of magnetic particle handling in microchannels is shown in Fig. 1a: the particles are trapped *via* an external magnet to form a 'plug,' different reagents are consecutively pumped through the plug. Once the desired reaction has been performed, the particles can be released for analysis or regeneration by removing the magnetic field. This concept has been demonstrated for nucleic acid hybridisation and recognition,[10,11,12,13] immunoassays,[14,15] and cell capture.[16] Despite advantages in terms of fast and efficient binding, this process is still comparatively time consuming. Typically, such procedures take between 30 and 60 mins to complete, since reagent and washing buffers must be flushed consecutively.

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Here, we present an innovative, dynamic concept for performing reactions on the surface of magnetic particles (Fig. 1b), by drawing particles through consecutive reagent streams. Due to laminar flow regimes in microfluidic devices, multiple reagent streams flowing in the *x*-direction can be generated across a chamber. Magnetic particles are introduced at one side of the chamber and a magnetic field applied perpendicular to the direction of flow. Particles experience a magnetic force in the *y*-direction and are deflected across the width of the chamber.[17] Thus, the deflected particles are pulled through the different flow streams in a controlled movement and chemical reactions or binding processes can take place on their surface. This permits procedures involving multiple steps to be carried out extremely rapidly since reactions, washing steps, particle isolation and detection are all performed in one single operation in continuous flow on a particle by particle basis. To demonstrate the viability of this concept we have performed a simple binding assay and associated washing steps.

The microfludic chip design featured four branched inlets, a rectangular reaction chamber 6 mm long and 3 mm wide and a single branched outlet (Fig. 2a). This design was suitable for

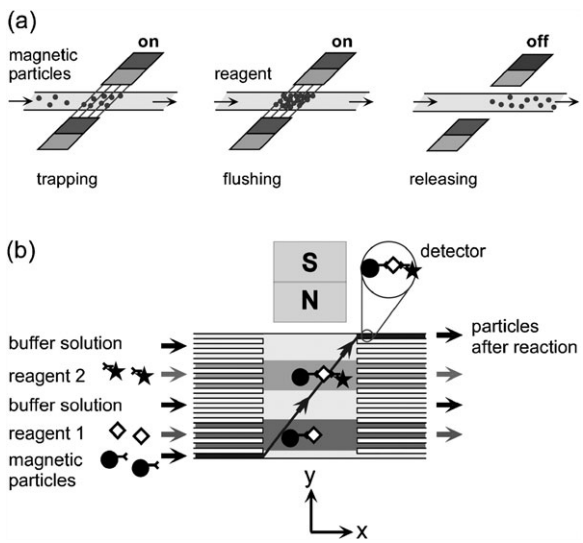

Fig. 1 (a) Surface chemistry on magnetic particles as performed conventionally: The particles are trapped and consecutively washed with reagents and washing buffers. (b) Here, a different principle is introduced whereby magnetic particles are continuously pulled through reagent streams. In the example shown above a sandwich assay is described, however, the same methodology could be applied to almost any surface based chemical process.

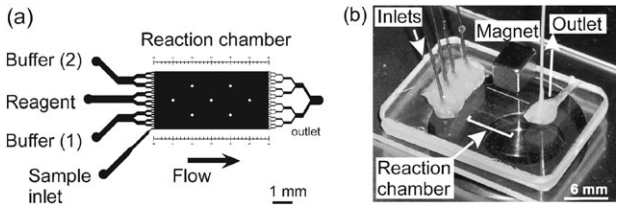

Fig. 2 (a) Schematic of the microfluidic chip design featuring a reaction chamber, four inlets and one outlet. (b) Photograph of the set-up showing the microfluidic chip and external magnet.

the introduction of particle suspension and three further liquid streams. The chip was fabricated in glass and etched to a depth of 20 μm. The magnetic field was generated by a permanent NdFeB magnet ($4 \times 5 \times 4$ mm^3) placed on top of the chip, to the side of the chamber. Microcapillaries (i.d. 150 μm, Metal Composites, UK) were glued into the inlets and interfaced to four 1 mL Hamilton syringes (Fig. 2b). Reagent and buffer solutions and magnetic particle suspensions were pumped under positive pressure *via* a syringe pump as required (Harvard Applications, USA). The magnetic particles (2.8 μm diameter, Dynal, Invitrogen) were streptavidin coated for the binding assay or plain epoxy coated for the negative test. The buffer used was 0.1 × TBE (0.1 M, pH 8.3, Sigma, UK) and the reagent was a solution of fluorescently labelled biotin (Molecular Probes, Invitrogen). The microchip was observed using an inverted fluorescence microscope (TE2000-U, Nikon, Japan) and images were captured using a CCD camera (WAT-221S, Watec, Japan) with the auto-adjustment disabled.

Initially, the generation of four parallel laminar flow streams was verified using alternating streams of yellow and blue ink such that the flow rate in the reaction chamber was 500 μm s^{-1}. Since equal pressure was applied to the four syringes, the fluid streams had an equal width of 750 μm.

For the deflection of magnetic particles,[17] streptavidin coated beads were pumped at a flow rate of 500 μm s^{-1} through the reaction chamber and the magnet was placed onto the device. The particles were deflected in the *y*-direction over the entire width of the chamber, crossing through the two central streams and leaving in the final stream. The force acting on the particle by the magnetic gradient was calculated to be in the pN range. A particle concentration of 6.7×10^6 beads mL^{-1} was used with an average particle throughput of 20 beads per min.

As a first example of a surface based biochemical process, a streptavidin–biotin binding assay was performed involving one binding step and two washing steps (Fig. 3). Streptavidin coated particles were introduced into inlet number 1, a buffer solution into inlet 2, a solution of fluorescently labelled biotin (1 μg mL^{-1}) into inlet 3 and a further buffer solution into inlet 4, so that the flow rate in the reaction chamber was 500 μm s^{-1}. The magnetic particles were pulled through the first buffer stream and the fluorescent biotin stream and into the final buffer stream. Under fluorescent light, unreacted particles exhibited slight background fluorescence, which permitted them to be tracked in dark conditions (Fig. 3a). As the particles passed into the biotin stream, they began to fluoresce more strongly. Their fluorescence increased in intensity as they traversed the stream (Fig. 3b). After the particles left the biotin stream and moved into the last buffer stream, they continued to fluoresce, indicating that biotin had bound and been retained on their surface (Fig. 3c). The experiment was repeated using epoxy coated magnetic beads. For these beads, no change in their fluorescence was observed after traversing the biotin stream, indicating that there was little or no binding to their surface.

The experiment with streptavidin coated beads was also performed using a biotin concentration of 0.2 μg mL^{-1} and the fluorescence intensity of the particles prior to entering the biotin stream and after leaving the biotin stream was compared. Images taken from videos were analysed using ImageJ software. The relative intensities of 12 particles before and after passing through the biotin stream were averaged and plotted against distance across the particle image (Fig. 4). The residence time of the particles within the biotin stream was approximately 15 s, which is considerably longer than would be expected from the applied flow rate of 500 μm s^{-1}. This was due to the placement of the magnet further towards the inlet end of the reaction chamber than the outlet end. Once the particles had passed the magnet, the field gradient was no longer perpendicular to the flow. Hence the magnet had a retarding effect on the particles, since at the point where the particles were in the biotin stream, the field was acting slightly in opposition to the flow. The time taken for each particle to cross the entire chamber was approximately 1 min. Reagent consumption of the system during an average 10 min experiment was approximately 3.6 μL of biotin and 13 μL in total including buffers and particle suspension.

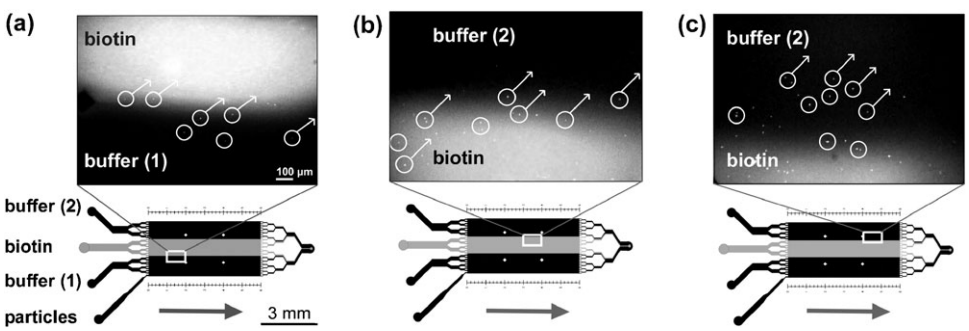

Fig. 3 Continuous flow binding, washing and isolation by pulling streptavidin coated magnetic particles through a stream of fluorescently labelled biotin into a buffer stream. (a) Particles before entering the biotin stream, (b) particles within biotin stream and (c) particles leaving biotin stream.

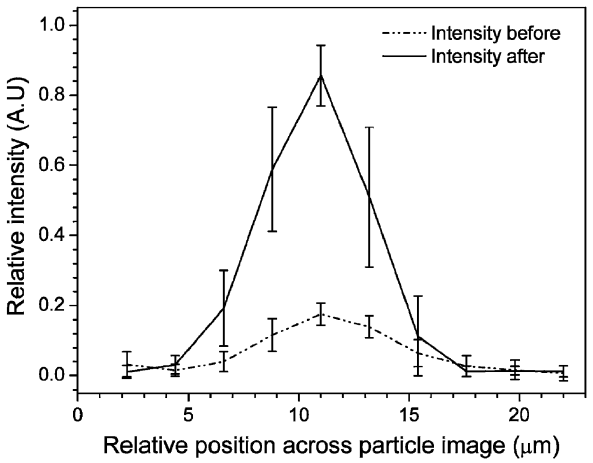

Fig. 4 Fluorescence intensity of particles before entering the biotin flow stream and after leaving the biotin flow stream.

The concentration of the biotin stream was 0.2 μg mL^{-1}, therefore during the average residence time each particle spent in the biotin stream it was exposed to approximately 1.3×10^{-17} mol of biotin. According to the information supplied by the manufacturer each particle could bind 9.7×10^{-18} mol, so there is potential for the system to detect lower biotin concentrations. However, our detection was limited by the use of a primitive CCD camera with poor sensitivity. Particles became difficult to observe at lower biotin concentrations, thus to increase the sensitivity of the assay and to obtain more comprehensive quantitative results, a more sophisticated camera such as an intensified CCD could be employed.

Inter-diffusional mixing between different flow streams is extremely important for this microfluidic system. This will depend on the diffusivity of the reagents being used, the flow rate and the length of the reaction chamber. Ideally, two reagent streams should be separated by a buffer stream that is at least as wide as the maximum inter-diffusional distances of the neighbouring reagent streams. For example, in this case, for fluorescently labelled biotin in aqueous solution (diffusivity $= 3.4 \times 10^{-6}$ cm^2 s^{-1})[18] in a 6 mm long reaction chamber and a flow rate of 500 μm s^{-1}, the maximum inter-diffusional distance at the end of the reaction chamber would be approximately 90 μm. Therefore, if there are two such streams separated by a buffer stream, then the buffer stream would have to be at least 180 μm wide to ensure the complete isolation of the two reagent streams.

These results successfully demonstrate the feasibility of fast sequential particle based (bio)chemistry in continuous flow. In one single operation over the course of approximately 1 min, the particles were isolated from the suspension liquid, mixed with reagent and then isolated from the reagent solution, washed and detected. This greatly reduces processing time from the 30 to 60 min in previously reported systems[10–16] to *ca.* 1 min.

In this example, only four flow streams were used, however, with a suitable magnetic field design, many more flow streams could be employed.[19,20] In addition to this, two or more devices could be connected in cascade, so that the particles from one device are fed into the next. Hence, multi-step chemical reactions, such as sandwich immunoassays, multi-step syntheses (DNA synthesis, peptide synthesis), drug screening or even the processing of magnetically labeled cells could be performed. Limiting factors include the reaction kinetics and the number of sample molecules present in the stream that can interact with each particle as it passes through the reagent stream. These limitations could be overcome by modifying the magnetic field such that the particles are either temporarily stopped in flow,[8] or are pulled to and fro through the reagent stream several times so that residence time is increased.

The controlled movement of particles is also not limited to magnetic forces. Many forces have been investigated for the deflection of particles in flow.[21] These include dielectrophoretic, acoustic, optical or gravitational forces.

The reagent streams used in this work were aqueous based. However, this platform is also suitable for mixed phase systems. Therefore the streams could be for example, alternately organic and aqueous, or another combination of both.[22–24]

A vast variety of chemical and bioanalytical reactions could be investigated in this fashion, by varying the surface chemistry of the particles and the composition of the reagent streams. Hence this novel, extremely versatile and dynamic system shows enormous potential for the fast and simple execution of multi-step processes and provides an alternative to the drudgery associated with many (bio)chemical procedures.

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