High-throughput sample introduction for droplet-based screening with an on-chip integrated sampling probe and slotted-vial array†‡

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We developed a droplet-based microfluidic screening system with an on-chip sampling probe integrating multi-channels for sample introduction, reagent merging and nanolitre-scale droplet generation, and a slotted-vial array sample presenting system. The present system was applied in protein crystallization conditions screening with an ultra-high sampling throughput up to 6000 h^{-1} for different samples.

In recent years, droplet-based microfluidic technology,[1–3] as one of the rapidly developing technologies in microfluidics, has shown its capabilities in producing isolated droplet micro-reactors and performing high-throughput screening analysis.[4–8] In most of the droplet-based microfluidic analysis systems, continuous flows of aqueous and oil phases are usually used to rapidly produce plenty of monodisperse droplets. The reagents are usually mixed with the sample before (pre-mixing) or after (post-mixing) the droplet generation, in which the continuous flow mode is also frequently adopted. However, one limitation of the continuous flow systems is that a special channel and reservoir are usually required for sample introduction, with which it is difficult to perform fast and convenient sample changing.

In high-throughput screening analysis, there are numerous different samples required to be screened. In such an application, how to introduce different samples into a screening system is of significance. So far, there are few reports on the development of sampling devices for multiple sample introduction in droplet-based systems. The cartridge technique[9–11] supplies an effective method for introducing different samples to a droplet-based system performing large-scale screening. Usually the cartridge is produced by sequentially aspirating different sample solutions and oil into a tube to form an oil-isolated droplet array. Reagents are introduced into these droplets by connecting the cartridge with a mixing device and delivering the segment flow out from the cartridge into this device, in which the sample droplets mix with the reagent by stream merging[9–11] or droplet fusion[8] methods. Beyond that, a potential possibility to achieve droplet-based screening for multiple samples is to employ microvalves and multichannel architecture[12–14] to generate droplets containing different samples and make these sample droplets fuse with reagent droplets. In these systems, each sample has its own sample introduction channel and microvalve to control the opening and closing of the channel. In addition, some integrated sampling probes were developed for droplet-based chemistrode systems to perform on-line sample introduction and droplet generation.[15,16]

In 2005, we developed a high-throughput sample introduction technique based on a capillary sampling probe and a slotted-vial array (SVA) sample presenting system, which has been successfully applied in microfluidic flow-injection and sequential injection analysis systems.[17–19] However, the sampling throughputs of these systems were limited to 300–1000 h^{-1} due to the significant dispersion of sample zones in the hydrodynamic continuous-flow systems which would lead to cross-mixing between adjacent samples when higher sampling frequencies were used. Recently, we developed an automated droplet-based analysis system based on a capillary and SVA system,[20] which was capable of automatically generating droplets with different sizes and compositions using a droplet assembling method under the control of a computer program. Since multiple components comprising the droplet were sequentially aspirated into the droplet, the throughputs of droplet generation are usually around 1000 droplets per hour.

In this work, we developed a droplet-based screening system based on an on-chip integrated sampling probe and SVA system to achieve automated sample introduction and droplet generation of different samples with ultra-high throughput up to 6000 h^{-1}. The on-chip sampling probe integrated multiple channel manifolds not only for sample introduction and droplet generation as in previously reported probes,[15,16] but also for multiple reagent merging, within a narrow probe tip region of 0.8 mm × 1.0 mm (width × length). The concept of the sampling probe integrating multiple manifolds partially came from the biomimetic design based on insect mouthparts, such as the mouthparts of bees[21] and mosquitoes[22] in which a merging channel and multi-channel structures are integrated, respectively. The actual demand on the use of the integrated sampling probe is to achieve rapid multistep liquid-handling operations within a limited space to obtain a high sampling throughput in a droplet-based microfluidic system.

The present droplet-based screening system consisted of the BEE chip, SVA sample presenting system, and two syringe pumps (Fig. 1a). The channel configuration of a BEE chip is illustrated in Fig. 1c and Fig. S1‡. This glass chip was fabricated using standard photolithographic, wet chemical etching and high-temperature bonding techniques as described elsewhere.[23]

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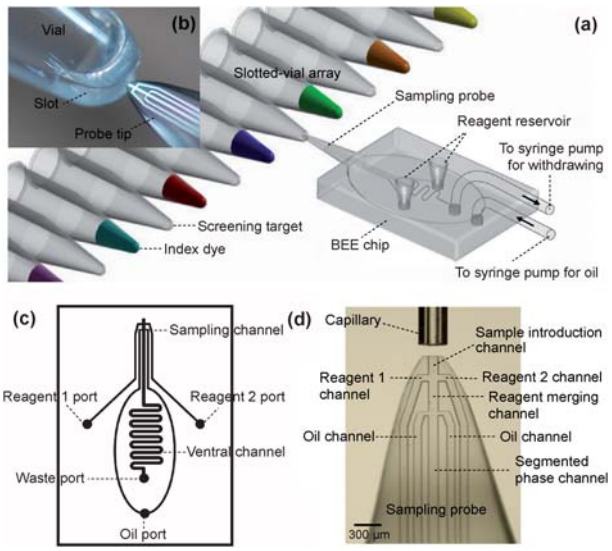

Fig. 1 (a) Schematic diagram of the setup of the droplet-based system consisting of the BEE chip with an integrated sampling probe and slotted-vial array (not to scale). (b) Close-up image of a slotted vial and the sampling probe tip. (c) Schematic diagram of the channel configuration in the BEE chip (not to scale). (d) Top view of the sampling probe tip and a fused-silica capillary (150 μm i.d., 320 μm o.d.) for comparison.

The on-chip monolithic sampling probe was fabricated as reported previously by the authors' group.[24,25] The tip size of the cone-shaped sampling probe was *ca.* 210 × 60 μm (width × thickness) (Fig. 1b,d). Before use, the outer surface of the probe and the chip channels were silanized with 4 mM octadecyl-trichlorosilane in n-heptane for 4 h at 25 °C. Two reagent reservoirs were made on the BEE chip surrounding the inlet ports of the reagent channels. The reservoirs were filled with aqueous reagents and provided liquid level pressures for reagent infusion in the microchannels. The slotted vials were made from 200-μL PCR tubes with a 1-mm slot produced at the bottom of each vial. The SVA was fixed on a translational stage with a step motor controlled by a computer program written with LabView. Two syringe pumps (PicoPlus 11, Harvard Apparatus, USA) were employed for liquid driving. One was connected to the waste port *via* a Tygon tubing for aspirating fluids into the chip ventral channel with a total withdrawal rate of $V_{withdrawal}$. The other one was connected to the oil port *via* another Tygon tubing for infusing oil into the chip channels with an infusion rate of V_{oil}. The sampling rate V_{sample} of the probe from the sample vial can be expressed by $V_{sample} = V_{withdrawal} - V_{oil} - V_{reagents}$.

The sample introduction was performed by immersing the sampling probe in the sample solution filled in the vial through the slot, and aspirating sample solution into the sample introduction channel of the probe at a sampling rate of V_{sample}. Within the sampling probe, under the pre-mixing mode, the introduced sample solution was first merged with the two reagent streams forming a multiphase laminar flow in the reagent merging channel, and then this aqueous flow was segmented into droplets by the oil streams (Fig. 2a). The sample was mixed with the reagents quickly within each droplets. In the present system, the sampling time for each sample was in the range of 0.45–3.0 s. The sample changing was conducted by linearly moving the SVA to switch the next sample vial to the sampling probe to aspirate

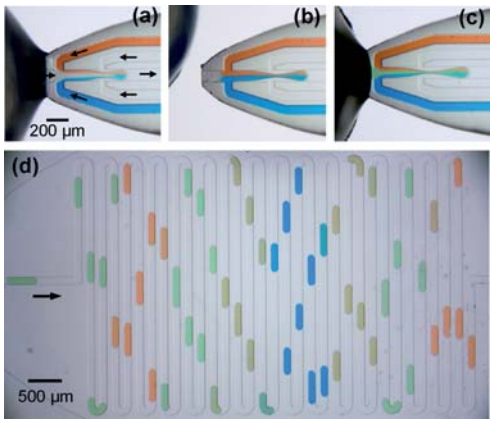

Fig. 2 Images of the sampling probe during the sample changing process of water and green dye solution with red and blue dye solutions as reagents. (a) Introduction of water. (b) Switching of the slotted vials. The carryover is less than 50 pL. (c) Introduction of green dye solution. The interval time between (a) and (c) is 0.15 s. (d) Image of the generated droplet array containing different dye samples in the ventral channel of the BEE chip. The arrows indicate the flow direction.

the new sample into the chip. The switching time between two adjacent vials was 0.15 s. By sequentially sampling from different vials, an array of droplets containing different samples for the subsequent reaction and screening analysis was formed in the ventral channel of the BEE chip.

The reason we adopted the pre-mixing method[10,26,27] for the introduction of sample and reagents and generation of droplets is that it has the advantages of simple system structure, convenient operation for mixing with multiple reagents and ensuring a fixed mixing ratio of sample and reagents before droplet generation. In the systems under post-mixing mode, either the stream merging[9–11] or droplet fusion[8] method is usually adopted to introduce reagents into the pre-formed sample droplets. The former method needs to match the reagent flow rates with the droplet frequency to ensure uniform ratio of sample and reagents in each droplet. The latter usually requires a special channel or device for droplet fusion, and accurate matching of the frequencies of sample and reagent droplets.

However, one limitation of the pre-mixing system is that a special channel and reservoir are usually used for sample introduction, with which it is difficult to perform fast and automated sample changing. This difficulty was overcome in the BEE chip by integrating the pre-mixing manifold into the monolithic sampling probe and coupling the probe with the SVA sample presenting system. By sequentially switching different sample vials in the SVA to the sampling probe, the sample changing operation could be achieved rapidly and conveniently.

The BEE system was first used to perform high-throughput introduction employing different dye solutions as model samples. Fig. 2 shows images of the sampling probe during the changing process of two samples (water and green dye solution), and an image of the generated droplet array containing different dye samples in the ventral channel of BEE. A droplet generation frequency of 3 Hz was obtained at a withdrawal rate of 800 nL min^{-1}. A sampling throughput of 1200 samples per hour was obtained with generation of *ca.* 9 droplets for each sample. The sampling time (*i.e.* residence time of the probe immersed in

sample solution) was 3 s, and the switching time between two adjacent vials was 0.15 s. As shown in Fig. 2b, when the vial removed from the probe, the carryover of the sample on the probe tip was less than 50 pL. Therefore, the cross-contamination between different sample vials was less than 0.01%, which could be omitted.

When the sample vial was switched to the next one, the sampling probe experienced a period outside the vials, *i.e.* exposed in air. This period was very short due to the fast sample-switching ability of the SVA system. Furthermore, the two reagent streams occupied the merging channel immediately after the vial removed from the sampling probe. Therefore, there was no air drawn into the microchannel during this period (Fig. 2b).

At the stage when the new sample vial was initially switched to the sampling probe, there was a cross-mixing phenomenon between the newly introduced sample and the previous sample remained in the sample loading channel as well as in the reagent merging channel, which would result in the generation of some transitional droplets sandwiched between the normal droplet series of the two samples. These transitional droplets usually could not be used in the subsequent analysis due to their uncertain compositions. If more transitional droplets are generated during the sample changing process, the sampling throughput of the system would be decreased evidently. In the BEE chip, benefiting from the design of the integrated sampling probe, an extremely short sample loading channel of 270 μm and reagent merging channel of 420 μm could be used in the probe. The cross-mixing between the two adjacent samples during the sample changing process was limited within this short channel region. Therefore, the sample changing operation could be completed quickly, producing as few transitional droplets as possible. In Fig. 2d, the sample changing between two adjacent samples was achieved within 0.7 s with only one transitional droplet formed between the normal droplet series.

An experiment was performed using almost the ultimate withdrawal rate of 9 μL min^{-1} in the present system to test the possible highest sampling throughput of BEE for different samples. A droplet generation frequency of ~24 Hz was obtained, and the throughput for different samples reached 6600 h^{-1}, generating 13 droplets for each sample (Fig. S3‡). Provided 8 droplets are required to be generated for analysis of each sample, the possible highest sampling throughput could exceed 10000 h^{-1}.

Moreover, due to the use of the pre-mixing mode, another important feature of BEE is that the mixing ratio of sample and reagents in each normal droplet can be maintained constant and independent of droplet volumes in fixed flow-rate conditions including $V_{withdrawal}$, V_{oil} and $V_{reagents}$. Therefore, no evident effect of the droplet volume variation during the droplet generation process on the subsequent screening was observed in the following experiments.

Fig. 3 shows a series of images of the sampling probe channel at different V_{oil}. At lower V_{oil} of 160 nL min^{-1} or 200 nL min^{-1}, a multiphase laminar flow formed between the streams of aqueous solutions and oil in the main channel, and it could even be maintained in the whole channel. In such a situation, no droplet formed in the channel due to the low shear stress of oil applying to the aqueous stream at the relatively lower V_{oil}. With V_{oil} higher than 240 nL min^{-1}, aqueous droplets could be continuously generated due to the increase in the oil shear stress with V_{oil}. When V_{oil} was higher than $V_{withdrawal}$, the oil entered the sampling and reagent channels, and prevented the sample solution from entering the sampling probe.

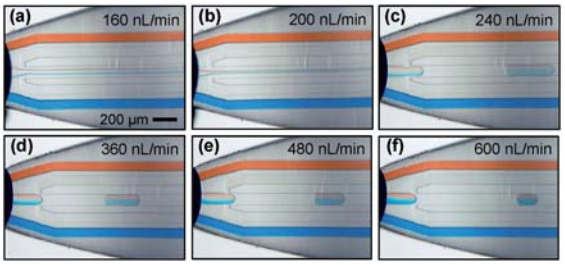

Fig. 3 Images of flow profiles in the sampling probe channel at different oil infusion rates of 160 (a), 200 (b), 240 (c), 360 (d), 480 (e), and 600 (f) nL min^{-1}, respectively. Sample, water; Reagents, red and blue dye solutions; Liquid levels of blue and red dye solutions in reservoirs, 1.0 cm above the chip; Total withdrawal rate, 600 nL min^{-1}.

In the BEE chip, the mixing ratio between sample and reagents in droplets depended on the ratio of V_{sample} and $V_{reagents}$, which could be readily adjusted by varying the liquid levels in the reagent reservoirs. The $V_{reagents}$ was determined by the $V_{withdrawal}$ of the syringe pump and the liquid levels in the reagent reservoirs. At a fixed $V_{withdrawal}$, the increase in the liquid level in the reagent reservoirs increased $V_{reagents}$, and thus decreased V_{sample}, leading to the decrease in the merging ratio of sample and reagents in the multiphase laminar flow as well as their mixing ratio in the subsequently formed droplets. Fig. 4 shows a series of images of the sampling probe channel with approximate merging ratios of *ca.* 1 : 6 : 1, 1 : 3 : 1, 3 : 1 : 3, 1 : 4 : 5, 5 : 4 : 1 (red : colorless : blue) corresponding to different liquid levels, respectively. The use of the gravity driving method for reagent solutions is in favor of reducing the number of syringe pumps used in the system, and this advantage is especially useful when more reagents (*e.g.* four reagents as in Fig. 4f) are required to be introduced. During the experiment process, the liquid levels in the reagent reservoirs dropped with the consumption of the reagents, which would lead to the decrease in $V_{reagents}$. Since $V_{reagents}$ was in the range of 100–300 nL min^{-1} in the present system, no evident effect of the liquid level variation on the $V_{reagents}$ was observed. For a prolonged screening period with

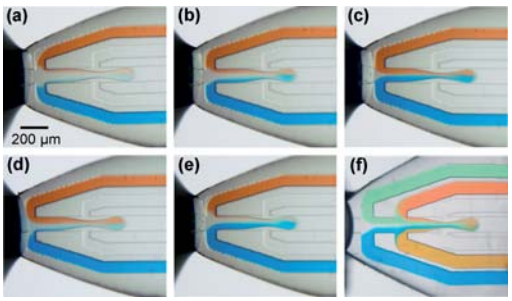

Fig. 4 Variation of mixing ratios of sample and reagents in the sampling probe with different liquid levels in reagent reservoirs of 0.5 and 0.5 cm (a), 1.0 and 1.0 cm (b), 1.5 and 1.5 cm (c), 0.5 and 1.5 cm (d), 1.5 and 0.5 cm (e) (blue and red dye solutions). (f) The sampling probe integrating four reagent channels. Other conditions are as in Fig. 3.

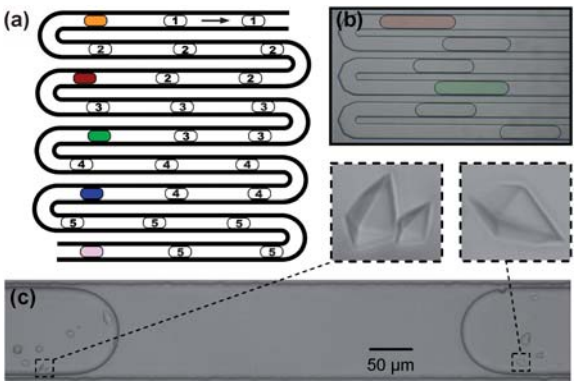

Fig. 5 Schematic diagram (a) and image (b) of the droplet series containing protein and different precipitants with different dye solutions indexing the droplet series. The marked droplet numbers are the index numbers for different precipitants. The arrow indicates the flow direction. (c) Microscopic images of thaumatin crystals obtained in droplets containing precipitant of 2.0 M potassium sodium tartrate in 0.1 M HEPES sodium salt, pH = 7.5.

large amounts of samples, larger reservoirs[28] or horizontal reservoirs[17,18] for reagents should be used to maintain stable liquid levels in them.

We applied the BEE system in protein crystallization conditions screening by using the different precipitants as samples, and the protein thaumatin and N-(2-acetamido) iminodiacetic acid buffer as reagents. Six droplet series were generated aiming to six precipitant conditions with 5–6 droplets in each series (Fig. 5a). The total sample introduction time for each precipitant was ~0.6 s including the vial switching time of 0.15 s and the precipitant introduction time of 0.45 s. The sample introduction from six different precipitant vials was achieved within 3.6 s, *i.e.* the sampling throughput for different samples could reach 6000 h^{-1}. Different dye droplets were sandwiched between the screening droplet series to index them (Fig. 5a,b). The indexing of droplets is an important issue for large-scale screening because usually it is difficult to distinguish the compositions of droplets from their appearances.[9,29] With the BEE chip coupled with the SVA system, the droplet indexing operation could be automatically achieved with the sample introducing operation by arranging the indexing dye vials between the sample vials in the SVA. After the generation of the droplet series, the BEE chip was incubated at 18 °C for 6 h. Thaumatin crystals were observed in the droplets containing precipitant solution of 2.0 M potassium sodium tartrate tetrahydrate in 0.1 M HEPES sodium salt (pH = 7.5) (Fig. 5c). In the droplets containing other precipitants, there were no evident crystals formed even after another 30-h incubation period. In this experiment, the consumptions of both the protein and precipitant were ~0.4 nL for each droplet, and ~2.4 nL for each condition screening. The extremely high throughput of droplet generation and reagent introduction, convenient droplet indexing, low sample and reagent consumptions make the BEE system especially suitable for large-scale screening with scarce samples or reagents.

In conclusion, the present droplet-based system with the integrated sampling probe has the advantages of high integrity, simple structure, fully-automated operation, extremely high sampling throughput for different samples, and low sample and reagent consumptions. It provides a novel approach to achieve automated and high-throughput sample introduction in droplet-based systems, and would have broad applications in high-throughput and large-scale chemical and biological screening analysis.

Although the BEE chip was made from glass in the present work, this technique is readily applied in microchips produced from other materials, such as polydimethylsiloxane (PDMS) or polymethyl methacrylate (PMMA) chips, in which the probe fabrication could be much easier by simply cutting the chip into a probe shape. In addition to droplet-based systems, the strategy of the sampling probe integrating multiple manifolds could also be applied in other microfluidic systems as an effective tool for sampling and sample pretreatment.

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