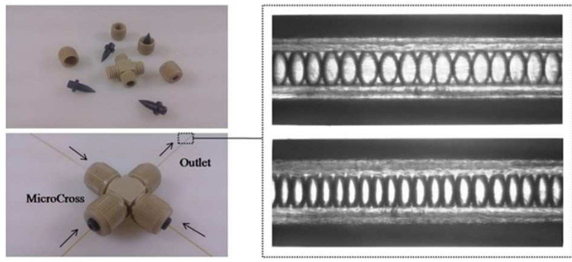
Lab on a Chip
RSC Publishing

We present a cheap, high pressure-resisting and easily-assembled device to produce micro W/O or O/W droplets of higher frequency.



Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/loc

Technical note

A 3D Easily-Assembled Micro-Cross for Droplet Generator

Ping Wu, Yong Wang, Zhaofeng Luo,Yuting Li, Mingfei Li and Liqun He*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

We present an off-the-shelf device combined by some commercial adapters to perform as a micro droplet generator. The advantage of this unit lies in that it is assembled conveniently, connected elegantly with other droplet detection equipments, and it is high-pressure enduring. Most important, this Micro-Cross circumvents the problem of hydrophilicity and hydrophobicity and produces W/O or O/W droplets at a higher frequency than PDMS chips.

Introduction

Micro droplets are recently widely used in biochemical analysis such as polymerase chain reaction (PCR)[1-3], multiple emulsions[4-6], drug delivery[7-9], microreactors[10, 11] and so on. Among the multifarious methods about droplet formation existed, microfluidic chips fabricated from flexible poly(dimethylsiloxane)(PDMS) through soft-lithograph[12] is one of the most popular methods characterized by its capability of producing homogeneous droplets/bubbles with a polydispersity index[13-15] λ< 2%. This on-chip droplet formation is further divided into three categories according to different flow regimes[16]: (1) formation in co-flowing streams[17], (2) formation in cross-flowing (T-junction) streams[18-20] and (3) formation in flow focusing elongational flowing streams[21, 22]. In these methods, droplet size distribution and generation frequency can be easily controlled by adjusting the flow rate. Besides, etching microchannels in glass or silicon are also used for droplet formation. Although techniques mentioned above have been proved quite successful, the need of high-level clean room, the tedious chip-fabrication processes, and the costly materials remain as barriers to the further promotion[4, 23]. And PDMS chips are not so suitable for droplet collection and storage, though latex tube is always used for liquid injection and outflow, droplet coalescence occurs inevitably at the connection between chip and tube. This problem must be considered in droplet PCR experiments especially when the droplets are drawn out for incubation by experiencing three stage of heating. Particularly, microchannel in PDMS chips is intrinsically hydrophobic and thus it is difficult to produce oil-in-water droplets unless surface wettability modification procedure is carried out.

Our goal is to report a simple method to form uniform micro droplets using an off-the-shelf device. We detail the commercial structure called Micro-Cross, instead of PDMS microfluidics, to perform as an alternative droplet generator. Connected directly with capillary tube, this device not only guarantees the monodispersity of droplets, but also applies to those high inlet pressure flow conditions to produce droplets of high-frequency. In addition, droplets generated from this micro device can be easily collected or manipulated in capillary tube, and the standardized capillary tube makes it rather convenient to connect with those droplet detection equipment, eliminating the problem of droplet coalescence. More important, unlike the 2D microfluidic channels, this 3D unit circumvents the problem of hydrophilicity and hydrophobicity, and 3D thread collapse is always faster than 2D collapse since it can be squeezed radially along any direction[24], resulting in higher emulsion frequency. Without high expense of ingredients and complicated processes as soft-lithograph, this off-the-shelf micro unit may be a fine choice for micro droplet formation.

Materials and methods

Micro-Cross construction

The same as the device used for particle focusing[23], our Micro-Cross (P-777, Upchurch Scientific) also contains several fluidic fitting parts such as MicroFerrule (F-152, Upchurch Scientific) and Female Nut (P-416, Upchurch Scientific) shown in Fig. 1(a). All of these adapters are from IDEX Health & Science, a part of IDEX Corporation designing, developing, and manufacturing liquid subassemblies and precision components. The detailed structure and internal geometrical dimension of cross-section of the device is illustrated in Fig. 1(c). Capillary tubes with 360 μm OD should be inserted through the PEEK(Polyetheretherketones) female nut and ferrules, pushed firmly against the bottom ledge. After finger tighten securely, this high-pressure-endurance unit should now be ready for use, whose original application is for mixing between different reagents in biotechnology applications. However, the fine capacity of generating micro droplets within this device, has not been developed before though it has been off-the-shelf for a long period. For those common Glass-PDMS, Plastic-PDMS or PDMS-PDMS microfluidic chips, plasma processing always achieves the best effect of bonding strength, yet the strength never exceeds 100 psi[25, 26]. Here, the device can withstand the maximum pressure of about 4000 psi, meaning that the demand of extremely high throughput is still satisfiable.

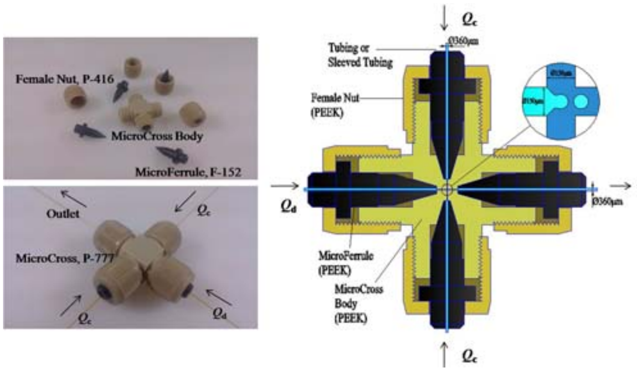


Fig. 1(a) High-pressure PEEK adapters including 4×P-416, 4×F-152 and the Micro-Cross body. **Fig. 1(b)** The assembled Micro-Cross P-777 connected by capillary tubing with 360 µm OD. **Fig. 1(c)** The cross-sectional view of the Micro-Cross, in which the channels are all three-dimension axisymmetric and the diameter of the core channels is 150 µm. Q_c and Q_d denote the flow rate of continuous liquid and dispersed liquid, respectively.

Table 1 Properties of continuous and dispersed liquids

Liquids	Density (kg/m^3)	Viscosity (Pa • s)	Oil-Water Interfacial tension (mN/m)
Mineral oil	863.7	24.44×10^{-3}	46.28
Silicon oil	905.2	9.05×10^{-3}	61.61
DI water	1000.0	1.00×10^{-3}	...
DI water + glycerol (1:1, v/v)	1175.8	53.71×10^{-3}	...

Droplet generator

As shown in Fig. 1(b), continuous phase liquid is injected symmetrically from two inlets and dispersed liquid enters from another, with the last one acting as an exit. At the location of the cross junction, the dispersed liquid is hydrodynamically focused to form a tip and eventually breakup into droplets due to natural instability. Flow-rate controlled syringe pumps (New Era Pump Systems Inc., USA) supply the power for liquid injection. Tab. 1 summarizes the properties of the liquids that we use in the present work, in which the viscosities are measured at 25^o C using a high precision viscometer (SYD-265H, Shanghai, China), and the interfacial tension between oil and water is carefully measured through the pendant-drop method.

In microfluidic chips, droplets are often confined to disk-like between top and down channel walls due to high aspect ratio of the chip, and this is accompanied by the problem of hydrophilicity and hydrophobicity during formation[4, 27]. Water-in-oil (W/O) emulsion process always fails in the hydrophilic channel whereas oil-in-water (O/W) dispersing system is suitable. Owing to that the dispersed phase liquid is squeezed radially without contacting the channel surface before breakup, this 3D axisymmetric channel is proved to be effective in forming monodisperse droplets without the need of water or oil wetting. As such, our device is reproducible in the emulsion of both W/O and O/W systems without considering the procedure of surface wetting. In W/O emulsion, we used mineral oil containing SPAN 80 surfactant (2.5%, w/v) as continuous liquid and deionized (DI) water as dispersed phase. In O/W droplets formation, we mix DI water with glycerol at a ratio of 1:1(v/v) as continuous phase, and low viscosity silicon oil is used as droplet phase. Hydrosoluble surfactant Pluronic F-127 (Jinsui Bio-Technology, Shanghai, China) is added to water to resist oil droplet coalescence.

Results and discussions

Formation of monodisperse W/O and O/W droplets

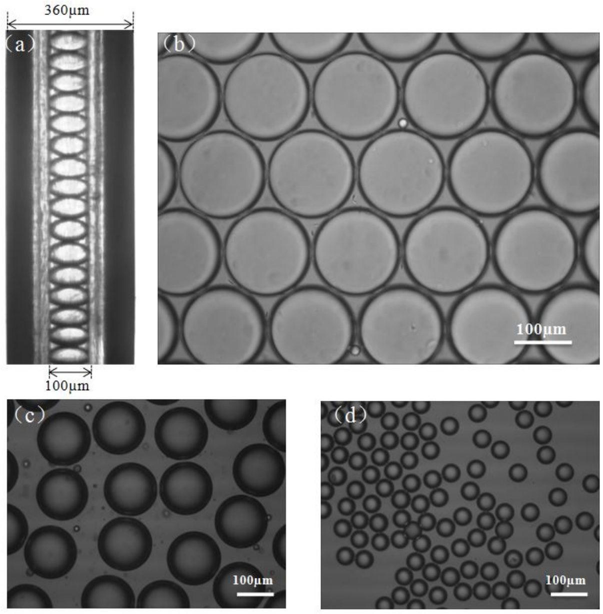


Fig. 2(a) Continuous W/O droplets in capillary tube with 360 µm OD and 100 µm ID. **Fig. 2(b)** Highly monodisperse W/O droplets, the mean diameter d_d is about 143.1µm, polydispersity index $\lambda\approx2.12\%$. **Fig. 2(c)** Highly monodisperse O/W droplets, flow rate of disperse phase Q_d=0.1 mL/h, flow rate of continuous liquid Q_c=0.3 mL/h, d_d=118.8 µm, $\lambda\approx$ 1.93%. **Fig. 2(d)** Smaller monodisperse O/W droplets in higher flow rate ratio, Q_d=0.1 mL/h, Q_c=3 mL/h, d_d=37.7 µm, $\lambda\approx2.89\%$.

We firstly use the Micro-Cross to demonstrate the generation of homogeneous W/O droplets as shown in Fig. 2(a-b). The existence of surfactant prevents the droplet coalescence though they are so in close proximity to each other in capillary tube in Fig. 2(a). Polydispersity index λ, defined as the ratio between stand deviation and the mean of these droplet diameter, is only about 2.12%, and this indicates that the droplets are highly monodisperse. Alternatively, the unit is also capable to generate uniform O/W droplets when silicon oil acts as dispersed phase, illustrated in Fig. 2(c-d). In this system glycerol must be mixed with water as continuous phase to increase the viscosity until it is comparable or higher than that of silicon oil. F-127 surfactant seems to form much thicker membrane around oil droplet surface against coalescence. Smaller droplets are generated as shown in Fig. 2(d), indicating that the flow rate ratio between continuous and dispersed liquid determines the droplet diameter distribution.

Droplet size distribution

Results in Fig. 3 shows both W/O and O/W droplet size decreases by increasing the flow rate ratio (Q_c/Q_d), and this tendency is consist with most emulsification process in chips. In those flow-focusing droplet formation microfluidic chips, the rate-of-flow-controlled breakup mechanism indicates that the droplet volume is inversely proportional to the flow ratio. In our experiments we firstly vary the flow rate of continuous phase Q_c at a constant flow rate, Q_d, of disperse phase, and then control the flow rate ratio to be constant, by simultaneously changing the flow rate of two liquids. It is observed that in 3D Micro-Cross the inversely-proportional relation between droplet size and flow rate ratio remains coincident with that of the 2D chips. Furthermore, higher flow rate ratio with higher flow rate of two phase produces smaller droplets. Thus we speculate the role of flow rate Q_c is most significant during the droplet breakup, owing to the fact that the pressure jump of continuous liquid at the entrance of downstream channel, caused by the occupation of disperse phase, leads to normal displacement of the thread and the eventual pinch-off[14].

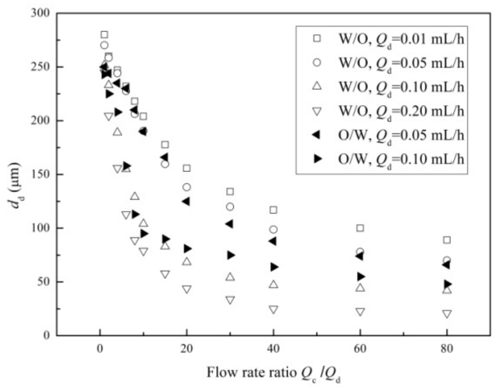


Fig. 3 W/O and O/W droplet size versus flow rate ratio.

Droplet generation frequency

In this section we show the difference of droplet formation frequency between the device described above and the traditional PDMS chips. The crossing chips are fabricated through soft-lithograph method with uniform depth 20 μm and the same size of width as the Micro-Cross (150 μm). Both the liquid properties and inlet flow rates are unaltered in the experiments and we observe that more droplets are generated during a same period in axisymmetric 3D Micro-Cross than the 2D rectangular cross-channel chips, shown in Fig. 4 and Fig. 5. As the theoretical analysis demonstrated[24], the 2D collapse is always stable whereas the 3D collapse is always unstable. The fluctuation of the dispersed phase thread, and the radial squeezing from outer fluid, accelerate the 3D breakup and thus higher generating frequency is achieved, especially in high flow rate ratio.

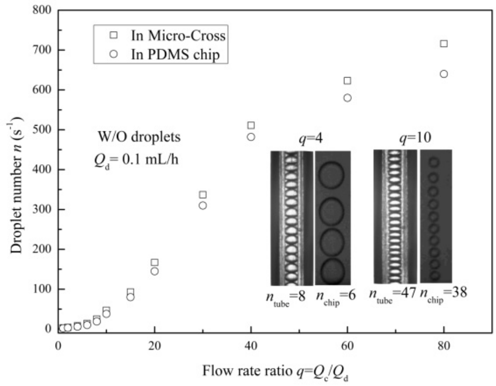


Fig. 4 Generation frequency of W/O droplets in Micro-Cross and PDMS chips. We obtain the droplet number n according to: $Q_d \Delta t = nV_d$, and we choose Q_d=0.1 mL/h, Δt=5 s, the droplet volume V_d is calculated from the measured mean droplet diameter d_d: $V_d = \pi d_d^3/6$.

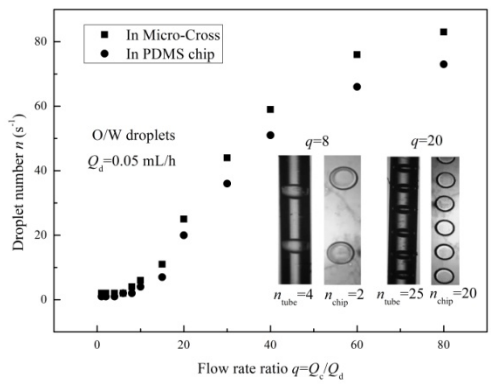


Fig. 5 Generation frequency of O/W droplets in Micro-Cross and PDMS chips. Q_d=0.05 mL/h, and the collection time is Δt=10 s.

Conclusions

As was illustrated here, the device is competent to generate micro W/O or O/W droplets without the need of channel surface wetting modification due to the 3D axisymmetric hydrodynamical focusing. Droplet size ranging from 20~300 μm can be easily controlled by altering the flow rate ratio, with polydispersity index lower than 3%. This unit withstands the highest fluid pressure (about 4000 psi) which is far higher than the chip-based microfluidic bonding strength (lower than 100 psi). Under the same material properties and flowing condition, the 3D Micro-Cross achieves higher generating frequency than the 2D PDMS chip with the same channel width. Although this adapter is designed for the occasional biotechnology applications when there is a special need for more than two pieces of tubing and high-pressure region systems, we develop its capability as a micro droplet generator by the comparative preponderance that this off-the-shelf device can be assembled easily, and valuable for the demand where both high throughput and low-cost are needed.

Acknowledgements

The authors would like to acknowledge the support from the

NSFC (50876100) and the Grade A Technology Development Foundation of USTC (ZC9850340103) and the IDEX Health & Science/Upchurch Scientific for supplying the detailed properties of the Micro-Cross.

Notes and references

Department of Thermal Science and Energy Engineering, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui, 230027, China. Tel: +86055163607146, E-mail: heliqun@ustc.edu.cn

1. M. Nakano, J. Komatsu, S. Matsuura, K. Takashima, S. Katsura and A. Mizuno, *J Biotechnol*, 2003, **102**, 117-124.
2. Y. Schaerli, R. C. Wootton, T. Robinson, V. Stein, C. Dunsby, M. A. A. Neil, P. M. W. French, A. J. deMello, C. Abell and F. Hollfelder, *Anal Chem*, 2009, **81**, 302-306.
3. Z. Zhu, G. Jenkins, W. H. Zhang, M. X. Zhang, Z. C. Guan and C. Y. J. Yang, *Anal Bioanal Chem*, 2012, **403**, 2127-2143.
4. N. N. Deng, Z. J. Meng, R. Xie, X. J. Ju, C. L. Mou, W. Wang and L. Y. Chu, *Lab Chip*, 2011, **11**, 3963-3969.
5. A. S. Utada, E. Lorenceau, D. R. Link, P. D. Kaplan, H. A. Stone and D. A. Weitz, *Science*, 2005, **308**, 537-541.
6. W. Wang, R. Xie, X. J. Ju, T. Luo, L. Liu, D. A. Weitz and L. Y. Chu, *Lab Chip*, 2011, **11**, 1587-1592.
7. K. Tang and A. Gomez, *J Aerosol Sci*, 1994, **25**, 1237-1249.
8. *Z. Z.* Wong and J. L. Bull, *J Drug Deliv Sci Tec*, 2011, **21**, 355-367.
9. Y. Kong, Y. Xu and F. E. H. Tay, *P Soc Photo-Opt Ins*, 2002, **4937**, 164-173.
10. L. M. Fidalgo, G. Whyte, B. T. Ruotolo, J. L. P. Benesch, F. Stengel, C. Abell, C. V. Robinson and W. T. S. Huck, *Angew Chem Int Edit*, 2009, **48**, 3665-3668.
11. H. C. Shum, A. Bandyopadhyay, S. Bose and D. A. Weitz, *Chem Mater*, 2009, **21**, 5548-5555.
12. J. C. McDonald and G. M. Whitesides, *Accounts Chem Res*, 2002, **35**, 491-499.
13. P. Garstecki, I. Gitlin, W. DiLuzio, G. M. Whitesides, E. Kumacheva and H. A. Stone, *Appl Phys Lett*, 2004, **85**, 2649-2651.
14. P. Garstecki, H. A. Stone and G. M. Whitesides, *Phys Rev Lett*, 2005, **94**.
15. Z. H. Nie, M. S. Seo, S. Q. Xu, P. C. Lewis, M. Mok, E. Kumacheva, G. M. Whitesides, P. Garstecki and H. A. Stone, *Microfluid Nanofluid*, 2008, **5**, 585-594.
16. G. F. Christopher and S. L. Anna, *J Phys D Appl Phys*, 2007, **40**, R319-R336.
17. P. B. Umbanhowar, V. Prasad and D. A. Weitz, *Langmuir*, 2000, **16**, 347-351.
18. P. Garstecki, M. J. Fuerstman, H. A. Stone and G. M. Whitesides, *Lab Chip*, 2006, **6**, 437-446.
19. J. D. Tice, H. Song, A. D. Lyon and R. F. Ismagilov, *Langmuir*, 2003, **19**, 9127-9133.
20. J. H. Xu, G. S. Luo, G. G. Chen and J. D. Wang, *J Membrane Sci*, 2005, **266**, 121-131.
21. S. L. Anna and H. C. Mayer, *Phys Fluids*, 2006, **18**.
22. S. L. Anna, N. Bontoux and H. A. Stone, *Appl Phys Lett*, 2003, **82**, 364-366.
23. A. Terray and S. J. Hart, *Lab Chip*, 2010, **10**, 1729-1731.
24. B. Dollet, W. van Hoeve, J. P. Raven, P. Marmottant and M. Versluis, *Phys Rev Lett*, 2008, **100**.
25. K. S. Lee and R. J. Ram, *Lab Chip*, 2009, **9**, 1618-1624.
26. S. Bhattacharya, A. Datta, J. M. Berg and S. Gangopadhyay, *J Microelectromech S*, 2005, **14**, 590-597.
27. Y. Morimoto, W. H. Tan and S. Takeuchi, *Biomed Microdevices*, 2009, **11**, 369-377.