Supplementary Material (ESI) for Lab on a Chip

Controllable Microfluidic Production of Multicomponent Multiple Emulsions

Supplementary Material

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Part S1. Experimental

S1.1. Fabrication of microfluidic device

The microfluidic device is based on three basic building blocks, as shown in Fig.1a. Cylindrical capillaries with outer diameter of 1.0 mm (AIT glass) are used as the injection tube and collection tube. The end of the injection tube is tapered by a micropuller (Narishige, Japan) and then adjusted by a microforge (Narishige, Japan). Square capillary tubes with inner dimension of 1.0 mm (Vitrocom, USA) are used to ensure the coaxiality of the cylindrical capillaries nested within them. For the drop maker, the injection tube and collection tube are aligned inside the square capillary tube, with the tapered end of the injection tube inserted into the collection tube. The coaxial co-flow geometry of drop maker is ensured by matching the outer diameters of the cylindrical capillaries to the inner dimensions of the square capillary tubes, as shown in Fig.S1. The dispersed fluid A is pumped into the injection tube, and the continuous fluid B is pumped through the outer coaxial region between the square capillary tube and the cylindrical injection tube. The coaxial flow of these two fluids results in the formation of monodisperse droplets in the collection tube. For the connector, two cylindrical capillaries are integrated to build a Y-shaped connector. For the liquid extractor, the injection tube and collection tube are aligned inside the square capillary tube but with the tapered end of the injection tube out of the collection tube. Fabrication of the microfluidic devices for various multicomponent multiple emulsions is achieved by selective bonding of the drop maker, connector and liquid extractor. For example, to fabricate the microfluidic device in Fig.1b, building blocks are bonded together by using a PVC tube to connect the cylindrical capillaries of every two building blocks, as shown in Fig.S2.

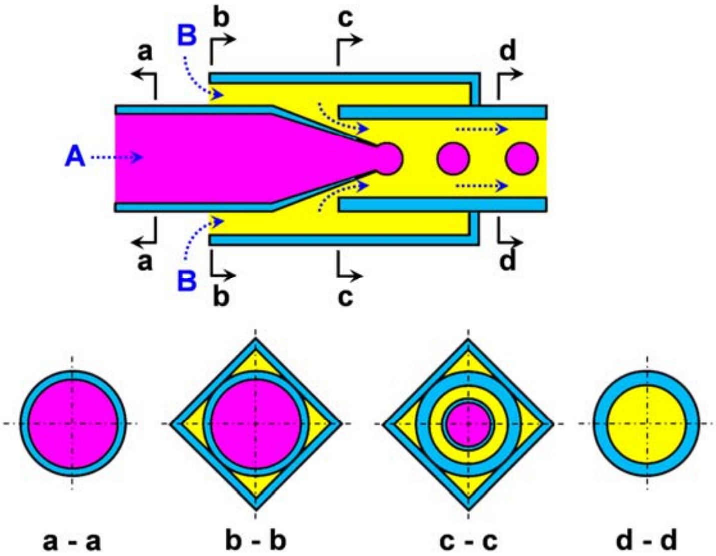

Fig.S1 Schematic diagram of the drop maker for generating monodisperse droplets. Illustrations a-a, b-b, c-c and d-d are cross-section images of the drop maker in relevant positions, which clearly show how the cylindrical capillaries and square capillary tubes are assembled in the drop maker. Fluid A is the dispersed phase, and fluid B is the continuous phase.

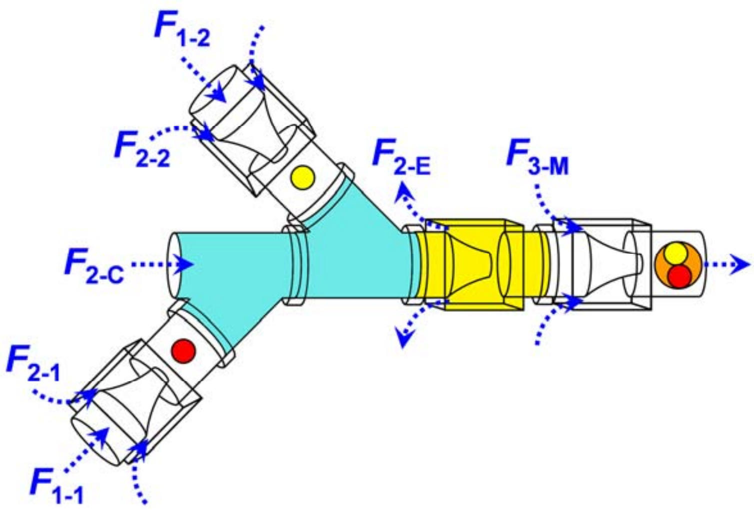

Fig.S2 Capillary assembly of the building blocks for the fabrication of microfluidic device for quadruple-component double emulsions. A PVC tube is used to connect the cylindrical capillaries of every two building blocks.

S1.2. Generation of multicomponent multiple emulsions

We generate multicomponent multiple emulsions by operating fluids of aqueous phase and oil phase into and out of the microfluidic device through syringe pumps. With 5 % (w/v) polyglycerol polyricinoleate (PGPR90, Danisco) as surfactant, soybean oil and benzyl benzoate are used as two kinds of oil phases. Aqueous solution containing 1 % (w/v) Pluronic F127 (Sigma-Aldrich) and 5 % (w/v) glycerol is used as aqueous phase. Either 0.1

% (w/v) Sudan red or 0.1 % (w/v) Sudan black is used to color the oil phase, and 0.5 % (w/v) methylene blue is used to color the aqueous phase.

For quadruple-component double emulsions, inner fluids $F_{1\text{-}1}$ and $F_{1\text{-}2}$ are soybean oil phase with and without Sudan red respectively, $F_{2\text{-}1}$, $F_{2\text{-}2}$, $F_{2\text{-C}}$ and $F_{2\text{-E}}$ are aqueous phases, and $F_{3\text{-M}}$ is soybean oil phase, as shown in Fig.1b. The variation ranges for the flow rates are $Q_{1\text{-}1} \approx 50\sim200$ μL h^{-1}, $Q_{1\text{-}2} \approx 60\sim300$ μL h^{-1}, $Q_{2\text{-}1} \approx 200\sim600$ μL h^{-1}, $Q_{2\text{-}2} \approx 300\sim700$ μL h^{-1}, $Q_{2\text{-C}} \approx 150\sim1600$ μL h^{-1}, $Q_{2\text{-E}} \approx 100\sim700$ μL h^{-1} and $Q_{3\text{-M}} \approx 4000\sim11000$ μL h^{-1}.

For quintuple-component double emulsions, $F_{1\text{-}1}$, $F_{1\text{-}2}$ and $F_{1\text{-}3}$ are benzyl benzoate oil phase labeled with Sudan red, Sudan black and without any dye respectively, $F_{2\text{-}3}$ is aqueous phase, and the other fluids are the same as those of quadruple-component double emulsions, as shown in Fig.4a. The variation ranges for the flow rates are $Q_{1\text{-}1} \approx 200\sim300$ μL h^{-1}, $Q_{1\text{-}2} \approx 200\sim300$ μL h^{-1}, $Q_{1\text{-}3} \approx 200\sim300$ μL h^{-1}, $Q_{2\text{-}1} \approx 500\sim600$ μL h^{-1}, $Q_{2\text{-}2} \approx 500\sim600$ μL h^{-1}, $Q_{2\text{-}3} \approx 500\sim600$ μL h^{-1}, $Q_{2\text{-C}} \approx 300\sim500$ μL h^{-1}, $Q_{2\text{-E}} \approx 200\sim700$ μL h^{-1}, and $Q_{3\text{-M}} \approx 4000\sim12000$ μL h^{-1}.

For quintuple-component triple emulsions, $F_{1\text{-}1}$ and $F_{1\text{-}2}$ are benzyl benzoate oil phase with and without Sudan red respectively, $F_{2\text{-}1}$, $F_{2\text{-}2}$, $F_{2\text{-C}}$ and $F_{2\text{-E}}$ are aqueous phases with methylene blue, $F_{3\text{-M}}$ is benzyl benzoate oil phase, and $F_{4\text{-M}}$ is aqueous phase, as shown in Fig.4c. The variation ranges for the flow rates are $Q_{1\text{-}1} \approx 300\sim350$ μL h^{-1}, $Q_{1\text{-}2} \approx 200\sim300$ μL h^{-1}, $Q_{2\text{-}1} \approx 850\sim900$ μL h^{-1}, $Q_{2\text{-}2} \approx 700\sim800$ μL h^{-1}, $Q_{2\text{-C}} = 300$ μL h^{-1}, $Q_{2\text{-E}} \approx 0\sim400$ μL h^{-1}, $Q_{3\text{-M}} \approx 1300\sim4000$ μL h^{-1} and $Q_{4\text{-M}} \approx 9000\sim13000$ μL h^{-1}.

For sextuple-component triple emulsions, $F_{1\text{-}1}$ and $F_{1\text{-}2}$ are benzyl benzoate oil phase with and without Sudan red respectively, $F_{2\text{-}1}$ and $F_{2\text{-}2}$ are aqueous phase and aqueous phase with methylene blue respectively, $F_{3\text{-}1}$, $F_{3\text{-}2}$, $F_{3\text{-C}}$ and $F_{3\text{-E}}$ are benzyl benzoate oil phases, and $F_{4\text{-M}}$ is aqueous phase, as shown in Fig.5a. The variation ranges for the flow rates are $Q_{1\text{-}1} \approx 0\sim400$ μL h^{-1}, $Q_{1\text{-}2} \approx 0\sim300$ μL h^{-1}, $Q_{2\text{-}1} \approx 400\sim1000$ μL h^{-1}, $Q_{2\text{-}2} \approx 350\sim1000$ μL h^{-1}, $Q_{3\text{-}1} \approx 1700\sim3600$ μL h^{-1}, $Q_{3\text{-}2} \approx 1700\sim3300$ μL h^{-1}, $Q_{3\text{-C}} \approx 300\sim500$ μL h^{-1}, $Q_{3\text{-E}} \approx 200\sim2000$ μL h^{-1} and $Q_{4\text{-M}} \approx 4000\sim20000$ μL h^{-1}.

Part S2. Results and Discussion

S2.1. Flow-rate-dependent control over number and ratio of different droplets in quadruple-component double emulsions

For quadruple-component double emulsions, the inner red and transparent droplets are separately formed from the drop makers in different branch channels, so the formation rate of each kind of droplets can be individually tuned by changing the relevant flow rates, which results in a regular array of red and transparent droplets with controlled ratio in the main channel, as shown in Fig.S3. When the formation rates of two kinds of droplets are equal, the red and transparent droplets arrange in the main channel one by one, where every one red droplet and one transparent droplet makes a periodic unit of the regular droplet array (Fig.S3(a)). Break-up in the intervals of periodic units leads to co-encapsulation of two kinds of droplets with controlled ratio at 1:1. By changing the flow rate of the outer fluid, every one, two and three periodic units can be encapsulated in double emulsions, resulted in quadruple-component double emulsions containing two, four and six droplets respectively, with the ratio (R_{12}) of the red droplets to transparent droplets fixed at 1:1 (Fig.S3(a)). By increasing the formation rate of the red droplets, regular arrays with more red droplets in every periodic unit can be obtained (Fig.S3(b)-(d)). Break-up in the intervals of these periodic units results in quadruple-component double emulsions with R_{12} controlled at 2:1, 3:1 and 3:2, as shown in Fig.S3(b), (c) and (d), respectively. Similarly, increase in the formation rate of the transparent droplets leads to regular arrays with more transparent droplets in every periodic unit (Fig.S3(e)-(g)), and periodic break-up of these arrays results in quadruple-component double emulsions with R_{12} controlled at 1:2, 1:3 and 2:3, as shown in Fig.S3(e), (f) and (g), respectively. Therefore, the number and ratio of the encapsulated droplets with different contents can be independently and precisely controlled by adjusting the flow rates of relevant fluids.

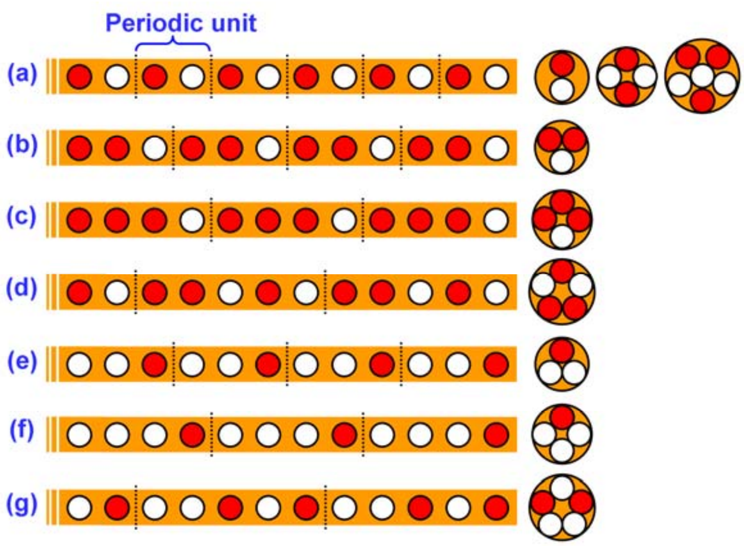

Fig.S3 Schematic diagram of controlled generation of quadruple-component double emulsions. Break-up of these regular droplet arrays in the intervals of periodic units can lead to co-encapsulation of two different droplets with precisely controlled number and ratio.

S2.2. Controlled generation of quadruple-component double emulsions containing two kinds of droplets with different sizes

The size of droplet can be tuned by changing the flow rates and/or inner the inner diameter (D_i) of the collection tube of drop makers. We illustrate the controllability of our microfluidic device over the size of co-encapsulated droplets by generating quadruple-component double emulsions that contain smaller red droplets and larger blue droplets (Fig.S4). Here we use drop makers with different D_i in branch channels to generate the smaller red droplets and larger blue droplets (Fig.1b). Also, the number and ratio of these two different droplets can be controlled by adjustment of the flow rate. The number of smaller red droplets increases from 0 to 5 while that of larger blue droplets keeps fixed at 1, resulted in a controlled ratio of smaller red droplets to larger blue droplets changing from 1:1 to 5:1, as shown in Fig.S4(b)-(f).

For generation of quadruple-component double emulsions containing smaller red droplets and larger blue droplets, the inner fluids $F_{1\text{-}1}$ and $F_{1\text{-}2}$ are benzyl benzoate oil phase with and without Sudan red respectively, $F_{2\text{-}1}$, $F_{2\text{-}2}$, $F_{2\text{-}C}$ and $F_{2\text{-}E}$ are aqueous phases, and $F_{3\text{-}M}$ is soybean oil phase, as shown in Fig.1b. The variation ranges for the flow rates are $Q_{1\text{-}1} \approx$ 0~350 μL h^{-1}, $Q_{1\text{-}2} \approx$ 250~750 μL h^{-1}, $Q_{2\text{-}1}$ =800 μL h^{-1}, $Q_{2\text{-}2}$ = 2000 μL h^{-1}, $Q_{2\text{-}C} \approx$ 1500~1800 μL h^{-1}, $Q_{2\text{-}E}$ = 200 μL h^{-1} and $Q_{3\text{-}M} \approx$ 4000~5000 μL h^{-1}.

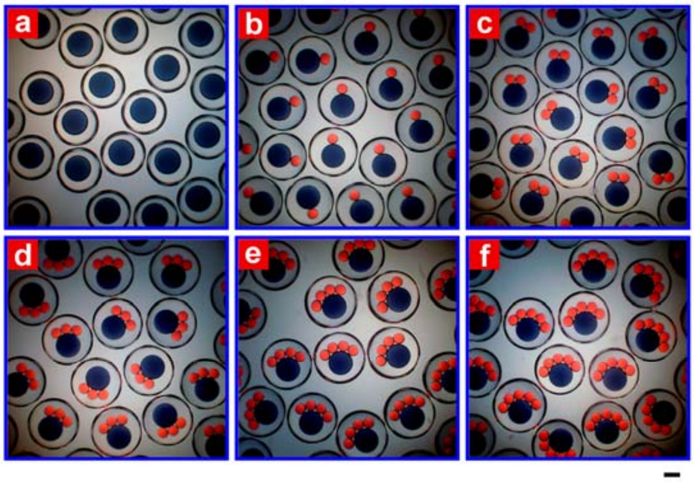

Fig.S4 Optical micrographs showing controlled production of quadruple-component double emulsions with the number of smaller red droplets increasing from 0 to 5 and that of larger blue droplets being fixed at 1. Scale bar is 200 μm.

S2.3. Independent control of connector and liquid extractor over the size of the outer droplets in quadruple-component double emulsions

Besides bonding the main channel with branch channels, another important functionality of connector lies in the adjustment of interval distance between droplets in the main channel. When we keep the other fluids fixed, the only increase in the injecting flow rate of connector leads to an increasing distance between every two droplets. To realize the encapsulation of two different droplets, it requires more continuous fluid that carries these different droplets to be sandwiched, resulting in an increasing size of the outer droplets (Fig.S5(a)). Similarly, the increase in the flow rate of liquid extractor leads to a decreasing distance between every two droplets. So, less continuous fluid needs to be sandwiched to realize the encapsulation of two different droplets, which results in a decreasing size of the outer droplets (Fig.S5(b)). Note that, because the inner droplets are first generated in branch channels, change of the flow rates in main channel will not affect the size of these inner droplets. Therefore, with combined functionalities of connector and liquid extractor, the size of outer droplets in quadruple-component double emulsions can be independently and precisely controlled.

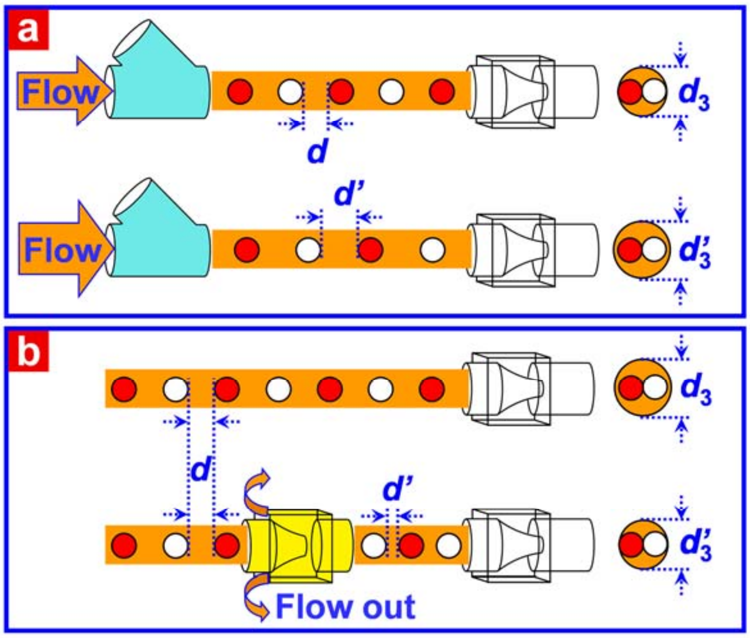

Fig.S5 Schematic diagram showing the flow-rate-dependent control over the size of outer droplets by connector (a) and liquid extractor (b). The distance between droplets can be adjusted by changing the flow rates of connector and liquid extractor so as to tune the size of outer droplets.

S2.4. Development of prediction equations for inner structures of quadruple-component double emulsions

For quadruple-component double emulsions, the total number (N) of both inner red and transparent droplets is controlled by matching the formation rates of inner red ($f_{1\text{-}1}$) and transparent droplets ($f_{1\text{-}2}$) with that of the outer droplets (f_3). According to mass conservation, the formation rate of each droplet can be described as follows:

$$f_{1-1} = \frac{Q_{1-1}}{\pi d_1^{\,3}/6} \tag{S1}$$

$$f_{1-2} = \frac{Q_{1-2}}{\pi d_2^{\,3}/6} \tag{S2}$$

$$f_3 = \frac{Q_3}{\pi d_3^{\,3}/6} \tag{S3}$$

Where $Q_{\text{i-j}}$ is the flow rate of fluid $F_{\text{i-j}}$; Q_3 is the sum of $Q_{1\text{-}1}$, $Q_{1\text{-}2}$, $Q_{2\text{-}1}$, $Q_{2\text{-}2}$, $Q_{2\text{-C}}$ and $Q_{2\text{-E}}$; d_{i} is the droplet diameter (as shown in Fig.1b). For fixed device dimensions (D_{i}) and solution conditions, the droplet diameter (d_{i}) in coaxial flow depends on the velocity of the surrounding flow in the dripping regime [S1], which can be described as follows:

$$\frac{d_1}{D_1} = a_1 \frac{Q_{1-1}}{Q_{2-1}} + b_1 \tag{S4}$$

$$\frac{d_2}{D_2} = a_2 \frac{Q_{1-2}}{Q_{2-2}} + b_2 \tag{S5}$$

$$\frac{d_3}{D_3} = a_3 \frac{Q_3}{Q_{3-\text{M}}} + b_3 \tag{S6}$$

Where coefficients a_{i} and b_{i} are the slopes and intercepts of these linear relations [S1]. Based on Equations (S1) to (S6), we can quantitatively predict the value of N using the following equation:

$$N = \frac{f_{1-1} + f_{1-2}}{f_3} = \frac{\dfrac{Q_{1-1}}{D_1^3\left(a_1\left(Q_{1-1}/Q_{2-1}\right)+b_1\right)^3} + \dfrac{Q_{1-2}}{D_2^3\left(a_2\left(Q_{1-2}/Q_{2-2}\right)+b_2\right)^3}}{\dfrac{Q_3}{D_3^3\left(a_3\left(Q_3/Q_{3-\text{M}}\right)+b_3\right)^3}} \tag{S7}$$

The ratio (R_{12}) of inner red droplets to transparent droplets is determined by the number of inner red and transparent droplets encapsulated in the double emulsions, which can be separately controlled by adjusting $f_{1\text{-}1}$ and $f_{1\text{-}2}$. Therefore, we can quantitatively determine the value of R_{12} using the following equation:

$$R_{12} = \frac{f_{1-1}}{f_{1-2}} = \frac{\dfrac{Q_{1-1}}{\pi d_1^{\,3}/6}}{\dfrac{Q_{1-2}}{\pi d_2^{\,3}/6}} = \frac{Q_{1-1}}{Q_{1-2}} \frac{D_2^3\left(a_2 \dfrac{Q_{1-2}}{Q_{2-2}} + b_2\right)^3}{D_1^3\left(a_1 \dfrac{Q_{1-1}}{Q_{2-1}} + b_1\right)^3} \tag{S8}$$

For fixed devices and emulsion systems, D_i, a_i and b_i are constant, so these equations for prediction of inner structure allow us to design multicomponent multiple emulsions with desired internals through fine adjustment of the flow rates. Moreover, prediction equations for higher-order multicomponent multiple emulsions can be further developed according to the mass conservation of each fluid phase.

References

[S1] L. Y. Chu, A. S. Utada, R. K. Shah, J. W. Kim and D. A. Weitz, *Angew. Chem. Int. Ed.*, 2007, 46, 8970.