Materials and methods Materials

An aqueous stock solution of 1×10^{-4} mol L⁻¹ rhodamine 6G (R6G) was prepared by dissolving 4.79×10⁻³ g R6G in 100 mL deionized water (DI water). A stock solution of 1×10^{-3} mol L⁻¹ R6G in hexyl alcohol was prepared by dissolving 4.79×10^{-2} g R6G in 100 mL hexyl alcohol. An etchant of 1 mol L⁻¹ HF - 1 mol L⁻¹ NH₄F was prepared by dissolving 16 mL HF and 14.8 g NH₄F in 400 mL DI water. Another etchant (1.5×10^{-2} mol L⁻¹ HF - 7.5×10^{-3} mol L⁻¹ HNO₃ - 7.5×10^{-3} mol L⁻¹ NH₄F) was prepared by dissolving 0.24 mL HF, 0.18 mL HNO₃ and 0.111 g NH₄F in 400 mL DI water. The acetone, ethanol, hexyl alcohol and other solvents used in this work were of analytical grade. The silanizing reagent (10%) was prepared by diluting 10 mL dimethyldichlorosilane in 90 mL hexyl alcohol. Glass substrate with chromium and AZ1805 photoresist coating (SG2056) were bought from Changsha Shaoguang Microelectronic Tech. Co., Ltd, China.

Apparatus

A mask aligner (MA/BA6, SUSS MicroTec, Germany) was used for the UV lithography. A water-bath shaker with temperature controller (THZ-82, Jintan Hongke Instrument Co., Ltd, China) was used for the etching. A surface profilometer (Dektak3 Series, Veeco Instruments Inc., US) was used to measure the depth of the micro/nanochannels. An AFM (SPA400, Seiko Instruments Inc., Japan) and SEM (LEO-1530, LEO Elektronenmikroskopie GmbH., Germany) were used to characterize the nanochannels. A contact angle measuring system (DSA-100, Kruss Gmbh, Germany) was used to measure the static contact angle.

A trinocular biological microscope (PH50-3A43L-A, Phoenix Optical Group Co. Ltd, China) with a CMOS image sensor was used to monitor the channels, and a personal computer with image processing software was used to control the CMOS image sensor. The microfluidic channel was connected to a precision syringe pump (LSP02-1B, Baoding Longer Precision Pump Co., Ltd, China) by polytetrafluoroethylene (PTFE) tubes to deliver the aqueous phase.

Fabrication of the hybrid microfluidic chip

Standard UV photolithography and wet chemical etching were used to fabricate the hybrid

microfluidic chip with micro/nanochannels on the glass substrate (as shown in Fig. S1). A V-shaped microchannel configuration on a photomask was transferred to the glass substrate during the first UV exposure. The microchannel was then etched by the etchant (1 mol L⁻¹ HF – 1 mol L⁻¹ NH₄F) at 40 °C. The depth of the microchannel was approximately 25 μ m after 10 min of etching, and the width was 400 μ m. The photoresist (BP212) was then spin-coated over the substrate again before a second UV exposure. During the second UV exposure, an I-shaped nanochannel configuration was also transferred onto the glass substrate. One end of the nanochannel exactly connected to the corner of the microchannels, and the whole channel looked like a Y-shaped configuration (as shown in Fig. 1a). The nanochannel was etched by the etchant (1.5×10⁻² mol L⁻¹ HF - 7.5×10⁻³ mol L⁻¹ HNO₃ - 7.5×10⁻³ mol L⁻¹ NH₄F). The depth of the nanochannel was 380 nm after 80 min of etching.

Room temperature bonding was used to bond the etched glass substrate to a glass cover. Three access holes were drilled at the ends of the channels and acted as the inlets and outlets. The etched substrate plate and cover plate were sequentially cleaned by a detergent and water stream. Finally, the two plates were bonded together under a continuous stream of water. The bonded plates were allowed to sit at room temperature for 2-5 days to complete the room temperature bonding process.

Results and discussion

Two-dimensional simulation

At the interface, the organic phase saturated the aqueous phase, and its concentration was constant (C). Because the aqueous phase flowed along the interface for less than several seconds, there was not enough time for the dissolved organic phase to reach the other wall of the microchannel. The unsteady diffusion in X axis can be described as follows:

$$\frac{\partial c}{\partial t} = \frac{v_1 \partial c}{\partial y} = -\left\{ \frac{j \Big|_{x + \Delta x} - j \Big|_x}{(x + \Delta x) - x} \right\}$$
(S1)

where c is the concentration of the dissolved organic phase at point x in the X axis, and j is the flux along the X axis. v_1 is the flow rate of the aqueous phase at the two-phase interface. If Δx is approaching 0, then:

$$\frac{v_1 \partial c}{\partial y} = -\frac{\partial j}{\partial x} \tag{S2}$$

If the diffusion coefficient (D) is constant, the following can be obtained by Fick's first law:

$$\frac{\partial c}{\partial y} = \frac{D}{v_1} \frac{\partial^2 c}{\partial x^2}$$
(S3)

If a new variate (ζ) is defined:

$$\zeta = \frac{x}{\sqrt{4\left(\frac{D}{v_1}\right)y}}$$
(34)

(84)

Equation S3 can be described as:

$$\frac{dc}{d\zeta} \left(\frac{\partial \zeta}{\partial y} \right) = \left(\frac{D}{v_1} \right) \frac{d^2 c}{d\zeta^2} \left(\frac{\partial \zeta}{\partial x} \right)^2$$
(S5)

Or
$$\frac{d^2c}{d\zeta^2} + 2\zeta \frac{dc}{d\zeta} = 0$$
 (S6)

Combined with the boundary conditions (when $\zeta=0$, c=C; when $\zeta=\infty$, c=0), equation S6 can be integrated:

$$\frac{c-C}{0-C} = erf\zeta = \frac{2}{\sqrt{\pi}} \int_0^\zeta e^{-s^2} ds \tag{S7}$$

The flux j at the interface (x=0), which is caused by the concentration gradient is:

$$j\big|_{x=0} = -D\frac{\partial c}{\partial x}\big|_{x=0} = C\sqrt{\frac{Dv_1}{\pi y}}e^{-\frac{x^2v_1}{4Dy}}\big|_{x=0} = C\sqrt{\frac{v_1D}{\pi y}}$$
(S8)

Thus, the total amount of the organic phase dissolved into the aqueous phase per second along the whole length of the interface is:

$$N = h \int_{0}^{l} j \Big|_{x=0} dy = Ch \sqrt{\frac{\nu_{1}D}{\pi}} \int_{0}^{l} \sqrt{\frac{1}{y}} dy = 2Ch \sqrt{\frac{l\nu_{1}D}{\pi}}$$
(S9)

where h is the depth of the nanochannel, and l is the length of the interface. Therefore, the average flow rate of organic phase (v₂) inside the nanochannel is:

$$v_2 = \frac{VN}{A} = \frac{2VCh}{A} \sqrt{\frac{lv_1D}{\pi}} = 2VC \sqrt{\frac{v_1D}{l\pi}}$$
(S10)

where V is the unit volume of the organic phase, and A is the cross sectional area of the nanochannel. If another new variate (k_1) is defined:

$$k_1 = 2VC\sqrt{\frac{D}{l\pi}}$$
(S11)

and equation S10 can be described as:

$$v_2 = k_1 \sqrt{v_1} \tag{S12}$$

The pressure driven flow in the microchannel has a parabolic laminar flow profile (as the blue lines in Fig. 1b). At the two-phase interface, the shear force drives the organic phase to flow in the Y direction. As the green rings in Fig. 1b, there is a circular flow in the organic phase. The right arm of the parabolic profile is elongated, and the the flow rate (v_1) at the interface is:

$$v_{1} = v_{\max} \left[1 - \left(\frac{r}{R}\right)^{2} \right]$$
(S13)

where r is the distance from the position with the flow rate of v_{max} in the microchannel to the interface, R is the distance from the position with the flow rate of v_{max} in the microchannel to the position with the flow rate of 0 in the circular flow. The v_{max} is the 2 folds of the average flow rate of the aqueous phase in the microchannel (v), so the equation S12 can be described as:

$$v_2 = k_2 \sqrt{v} \tag{S14}$$

where the variate (k₂) is defined as:

$$k_2 = 2VC \sqrt{\frac{2D}{l\pi} \left[1 - \left(\frac{r}{R}\right)^2 \right]}$$
(S15)

Fabrication and characterization of the nanochannel

Many methods have been developed to fabricate nanochannels, including focused ion beam (FIB) [1], nanoimprint lithography (NIL) [2, 3] and electron beam lithography (EBL) [4, 5]. These techniques enable the precise fabrication of one-dimensional or two-dimensional nanostructure. However, these techniques are expensive or difficult to scale up for mass production. In this study, two-step photolithography combined with wet chemical etching and room temperature bonding was used to fabricate hybrid microfluidic chips with micro/nanochannels [6]. After the first UV exposure, the etchant with the normal concentration was used to fabricate a channel with a depth in micrometers. Then, the photoresist was again spin-coated over the glass substrate. The etchant of a diluted concentration was used to prepare the channel with a depth in nanometers after the second UV exposure. Room temperature bonding was adopted to prevent the nanochannel from collapsing during bonding.

Compared with the microchannel, the etching was more critical for the nanochannel. If the etchant with the normal concentration was used (the etching rate was approximately 2.5 μ m min⁻¹), it only took several seconds to etch a nanochannel with a depth in nanometers. Therefore the diluted etchant was used during the preparation of the nanochannels. As shown in Fig. S2a, the effect of the different concentrations of the etchant was studied (the ratio of HF:HNO₃:NH₄F was maintained at 1:0.5:0.5). The depth of the channel increased from 93.4±2.1 nm to 1311.1±15.5 nm when the HF concentration increased from 1.5×10⁻² mol L⁻¹ to 6.0×10⁻² mol L⁻¹ after 20 min of etching. The coefficient of determination was 0.9986.

The etching time was also studied, as shown in Fig. S2b. The etchant with a concentration of 1.5×10^{-2} mol L⁻¹ (in the term of HF) was used because it provided the nanochannel with a depth from tens to hundreds of nanometers. When the etching time increased from 5 min to 90 min, the depth of the nanochannel increased linearly from 21.0 ± 1.6 nm to 402.4 ± 10.6 nm with a coefficient of determination of 0.9978. Under these conditions, the etching rate was calculated to be 4.68 nm min⁻¹. Except where otherwise stated, the depth of the nanochannel used in this work was 400 nm after etching for 90 min. The solvent's dissolution and the fluidic control method can also be realized in the nanochannel with a depth less than 100 nm, but it was difficult to illuminate the solvent's flow inside the nanochannel because of the short path of the light.

After etching, the depth of the nanochannel was measured to be 394 nm by the surface profilometer. AFM and SEM were also used to characterize the surface topography of the nanochannel. The edge of the nanochannel was scanned by AFM, and the side-view and top-view of the nanochannel are shown in Fig. S3a and S3b. From the AFM images, the etched area was obviously lower than the un-etched area in height. The roughness of the above areas was also measured. Before etching, the arithmetic average (Ra) of the roughness was 0.7229 nm, whereas the Ra was 0.9524 nm after etching. The etching increased the Ra by 31.7%. Compared with the depth of the nanochannel (~400 nm), the roughness increase caused by the etching was negligible. Fig. S3c and Fig. S3d show the SEM images of the nanochannel, with magnification factors of

300 and 10000, respectively. The edge of the nanochannel curved in the range of 1~2 micrometers, which was caused by the resolution of the photomask (25400 dpi) and the UV diffraction during the exposure. After the room temperature bonding, the microfluidic chip was cut across the nanochannel and scanned by SEM again. The SEM images of the cross section are shown in Fig. S4b-d, with magnification factors of 10000, 30000 and 50000, respectively.



Fig. S1 The fabrication of the hybrid microfluidic chip.



Fig. S2 (a) The relationship between the channel's depth and the etchant concentration (in terms of HF). The ratio of HF-HNO₃-NH₄F in the etchant was 1:0.5:0.5. The etching was performed for 20 min at 40 °C. (b) The relationship between the channel's depth and the etching time. The etchant contained 1.5×10^{-2} mol L⁻¹ HF, 7.5×10^{-3} mol L⁻¹ HNO₃ and 7.5×10^{-3} mol L⁻¹ NH₄F.



Fig. S3 Side-view (a) and top-view (b) of the edge of the nanochannel. The depth of the nanochannel was approximately 400 nm. (c) and (d) show the SEM images of the nanochannel. The magnification factors are 300 and 10000, respectively.



Fig. S4 The depth of the nanochannel measured by the surface profilometer before bonding. (b), (c) and (d) are the SEM images showing the cross section of the nanochannel after bonding; the magnification factors are 10000, 30000 and 50000, respectively.



Fig. S5 Without the two-phase interface at the junction of micro/nanochannels, the solvent in the nanochannel was driven to flow toward the other end of the nanochannel. (b) was taken 20 seconds after (a). Both images were taken at the position which was indicated by the red circle in the inset. A section of aqueous phase (the white area in the nanochannel) was introduced into the organic phase (the purple area in the nanochannel) to show the flow inside the nanochannel.



Fig. S6 (a) The relationship between the dimethyldichlorosilane concentration and the DI water contact angle. (b) The relationship between the treatment time and the contact angle.



Fig. S7 (a) The contact angle of DI water before and after the modification. The contact angle increased from $31.29^{\circ}\pm0.98^{\circ}$ to $83.32^{\circ}\pm0.85^{\circ}$. (b) The contact angle of hexyl alcohol before and after the modification. The contact angle decreased from $25.07^{\circ}\pm0.42^{\circ}$ to $19.21^{\circ}\pm0.86^{\circ}$. (c) The effect of the selective modification on the interface. Without the modification, the aqueous phase flowed into the nanochannel after several minutes. After the modification, a stable interface was generated, and the aqueous phase did not flow into the nanochannel. The solid and dashed arrows show the flow direction of the aqueous and organic phases.



Fig. S8 The fluorescence quenching of fluorescein under different pH conditions. (a) pH=2.12, (b) pH=3.31, (c) pH=4.58 and (d) pH=5.80.

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