Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

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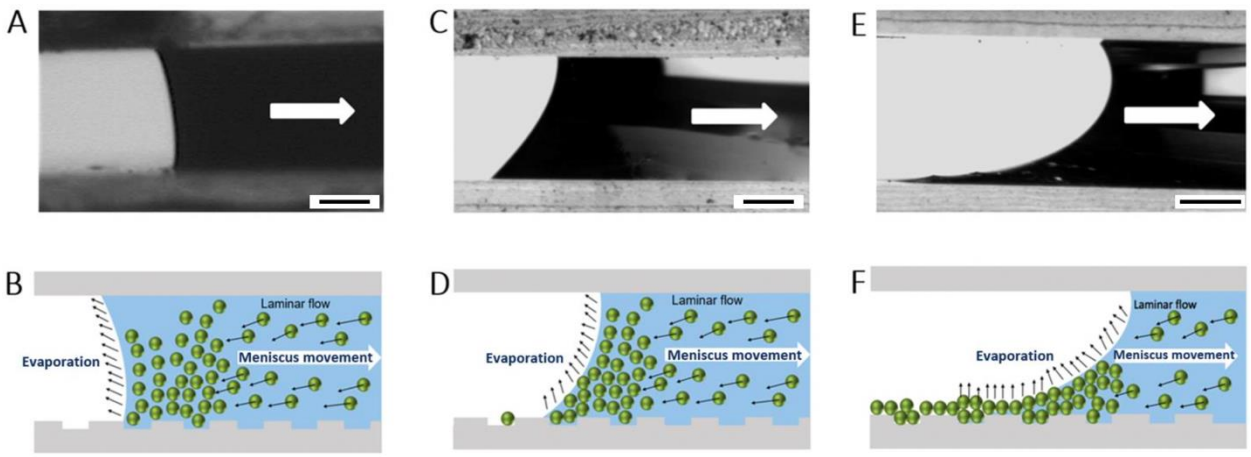
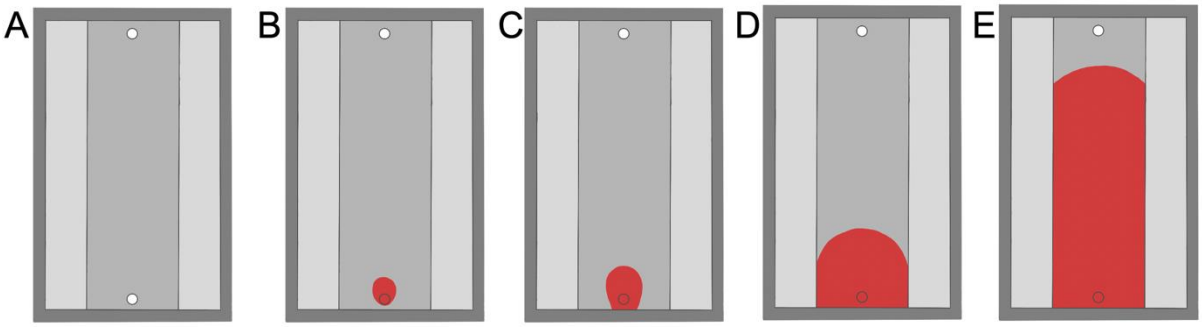
Contact angle. The front particles in contact with the air–liquid interface at the template level experience a downward capillary force F_c perpendicular to the meniscus. This force partly transmits to the rear particles, generating an upward force F'_c, which pushes them out of the traps (Fig. 2). This results in only the front particles being trapped during one deposition step, one for each trap. In order for this mechanism to work properly, a receding contact angle between 30° and 60° is optimal. Above 60°, the downward capillary force decreases, significantly affecting the yield. At the limiting case for an angle of 90° the force is zero, corresponding to a null yield (Supplementary Figure 1A, B). A receding contact angle between 30° and 60° grants the highest yield, with single particles trapped in each well after one deposition (Supplementary Figure 1C, D). At contact angles below 30°, the mechanism that enables the trapping of individual particles for each deposition no longer acts. In the extreme case, for a sufficiently small angle, crystals are deposited on the template (Supplementary Figure 1E, F). The value of the angle can be tuned to fall within the desired interval, since it is a function of the surface properties of the PDMS and the surface tension of the liquid. In the case of an untreated PDMS surface, the main parameter to adjust the contact angle, by modulating the surface tension of the fluid, is the concentration of surfactant in solution in the colloidal suspension. A concentration of 0.015% v/v TWEEN 20 in colloidal suspensions with 0.1% particles gives a receding angle that falls within the desired interval, for colloidal suspensions of both 1-μm and 2-μm diameter particles. The concentration of surfactant in solution is therefore sufficient as a control parameter for the contact angle, so that it is not necessary to treat the PDMS surface. The contact angle measurements in Figure 1A, 1C and 1E were done using a channel whose walls had been previously cut and removed to grant lateral optical access. The channel was set on the stage of an instrument for contact angle measurement (Krüss DSA 100), with a lateral camera for visualization. The solution was first injected into the channel and then pulled using a syringe controlled by a syringe pump with a 0.1 μL/min flow rate.



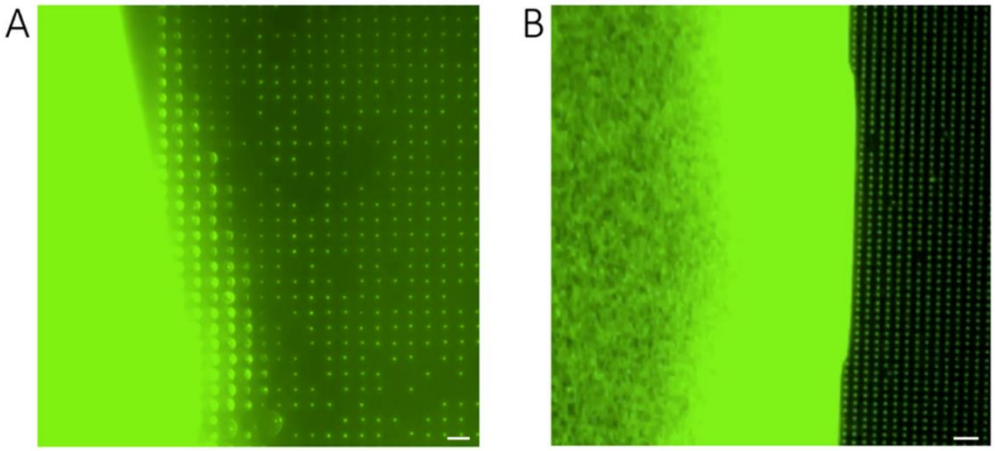
Supplementary figure 1: The success of particle deposition depends on the receding contact angle of the suspension's meniscus. (A-C-E) Photograph (side view) of the central section of the channel without lateral walls to enable optical access. The scale bar is 200 µm. (B-D-F) Corresponding schematics of the particles in the accumulation zone. The scheme is not in scale. (A-B) The receding contact angle is >60°. The schematic side view of the channel shows that the yield collapses because the capillary force directed downwards, which is responsible for the particle trapping, is greatly reduced. (C-D) The receding contact angle is within the optimal range, i.e. between 30° and 60°. The schematics shows the optimal scenario for particle deposition, where single particles are trapped. (E-F) The receding contact angle is <30°. The schematic side view shows the formation of colloidal crystals, which are released onto the substrate, due to the direction of the evaporation front, as in the case of convective assembly (Ref 17, Main text).

Channel-filling process. The liquid suspension is first manually injected into the channel through the inlet hole, located within the central section (Supplementary figure 2A), using a syringe. Once the liquid enters the channel (Supplementary figure 2B), it gets immediately in contact with both the floor and the ceiling of the central section (500 µm high). The liquid suspension is then gradually pushed and keeps spreading radially (Supplementary figure 2C) within the middle section until it laterally reaches the sides of the central section (Supplementary figure 2D). Once the suspension has reached the sides of the central section, surface tension prevents the liquid from spreading into the lateral sections, which present a greater (i.e. 1 mm-high) gap, and the meniscus proceeds forward along the central section with a convex-shaped front (Supplementary figure 2E). Once the template is covered, the injection process is completed and the liquid can be withdrawn using the syringe pump, thus starting the deposition process. Following the same principle, the two lateral air pockets are stably maintained when the liquid suspension is pulled during the deposition process.



Supplementary figure 2: Schematic of the channel-filling process. (A) Top view of the empty channel. The darker central region has a lower height than the two side lighter regions. (B) The liquid suspension is injected through the inlet hole, located in the central section of the channel. The liquid suspension gets immediately in contact with the ceiling and the bottom of the central section. (C) The liquid suspension is pushed into the channel and keeps spreading radially. (D) The liquid suspension reaches the sides of the central section, without spreading into the lateral sections thanks to surface tension and the height difference between central and lateral sections. (E) The liquid suspension proceeds forward, along the central section, with a convex-shaped meniscus.

Condensation. The temperature in the channel must be maintained above the dew point of water in order to avoid condensation on the template. This can be achieved by setting the assembly temperature at 27 °C to 30 °C, approximately 15 °C above the dew point of water. However, maintaining the average temperature in the channel above the dew point is not sufficient. It is also essential to maintain a uniform temperature on the template, and particularly in the region close to the air–liquid interface. This region is characterized by a high vapor concentration in the air, and even minimal temperature gradients on the template can lead to condensation. Condensation on the template is highly undesirable because it hampers the controlled deposition of particles in the traps. In the optimal scenario, where no condensation occurs, the deposited particles are in contact with the walls of the traps during the first deposition, and in contact with the previously deposited particle in the case of sequential deposition. If condensation occurs, however, the assembled particle can remain suspended within a drop of liquid inside the trap after deposition, and only come into direct contact with the PDMS trap after the drop has evaporated (Supplementary figure 3A). This causes the deposition position of the particle within the trap to be random, thereby losing the controllability of the assembly process. The condensation phenomenon can also lead to collapse of the deposition yield, since the necessary conditions for the assembly mechanism would not occur. The heated glass plate (Okolab H601-NIKON-TS2R-GLASS), placed underneath the microfluidic channel, guarantees a uniform temperature along the template, preventing condensation in the vicinity of the air–liquid interface.



Supplementary figure 3: Condensation on the channel's floor is determined by local temperature gradients in the vicinity of the accumulation zone. (A) The microfluidic channel has been placed into a heated chamber (Okolab H301-K-Frame) with a uniform inside temperature, set at 30°C. The chamber heats from the lid and from the walls but not from the base, located underneath the channel. The temperature is therefore not uniform on the channel's floor, thus generating local temperature gradients that, due to the high humidity close to the air-liquid interface, cause condensation on the template. The scale bar is 15 µm. (B) The channel has been placed onto a heated glass plate (Okolab H601-NIKON-TS2R-GLASS) that uniformly heats the channel, thus achieving a uniform temperature on the channel's floor (30°C). The absence of local temperature gradients on the channel's floor, and a temperature above the dew point of water, prevents any condensation on the template, granting particles to be correctly deposited into the template's traps. The scale bar is 25 µm.

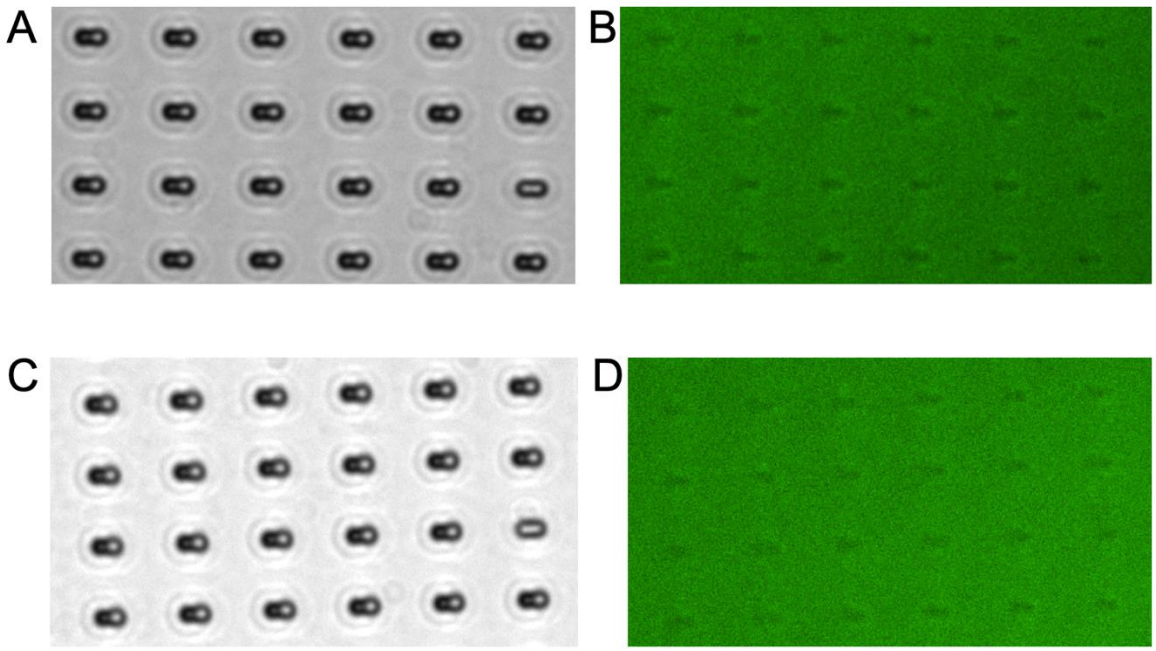
Deposition step 1 – Yield the first green particle (G)	
G (green)	74.8
No particles	25.2
Total yield	74.8

Deposition step 2 – Yield of a red particle (R)	
GR (green – red)	73.8
R (red)	24.3
No particles	1.0
Total yield	98.1

Deposition step 3 – Yield the second green particle (G)	
GRG (green – red – green)	51.9
RG (red – green)	35.5
No particles	0.95
Total yield	87.4

Supplementary table 1: Yields of the sequential depositions for the formation of a two-dimensional colloidal array of particle trimers. In Figure 4, the yield of specific particle sequences after each deposition steps are reported. In this Table, the yield of each deposition step, which can be obtained by summing the yield of all the sequences in which a new particle was deposited, is reported.

Control experiment for biotin-streptavidin. A negative control experiment was performed in order to prove that the biotin dye does not bind to non-functionalized trapped particles. We trapped non-functionalized, non-fluorescent polystyrene of 1 µm in diameter in 2-µm long, 1-µm wide, and 500-nm deep traps and followed the same functionalization protocol. Specifically, we filled the channel with a solution of green fluorescent biotin dye (10 µM Atto 520-Biotin and 10 mM PBS). The solution was left in the channel for 10 h and then flushed away with water to image the template. The template was imaged before (Supplementary figure 4A, B) and after (Supplementary figure 4C, D) filling the channel with the biotin dye solution. Bright-field (Supplementary figure 4A, C) and epifluorescence (Supplementary figure 4B, D) images of a section of the PDMS template were acquired. The biotin molecules do not bind to the polystyrene particles, which therefore show no fluorescence.



Supplementary figure 4: The fluorescent biotin dye does not bind to non-functionalized trapped particles. (A, B) Bright-filed (A) and epifluorescence (B) image of a section of the PDMS template after a single deposition of non-fluorescent non-functionalized polystyrene particles. The traps contain one particle each, except for the trap in the third row, and sixth column, that is empty, in order to show the visual difference between filled and empty traps. **(C, D)** Bright-field (C) and epifluorescence image (D) of the same PDMS template section, after filling the channel with a biotin fluorescent dye solution. The biotin molecules do not bind to the particles that thus keep showing no fluorescence.