Supporting Information for

SlipChip for immunoassays in nanoliter volumes

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Reagents and Materials. Reagents purchased from commercial sources were used as received unless otherwise stated. Soda–lime glass plates with chromium and photoresist coating were purchased from Telic Company (Valencia, CA). OmniCoat, SU8 2025, and Remover PG were purchased from MicroChem Corp. (Newton, MA). MF CD26 was obtained from Rohm & Haas Electronic Materials (Marlborough, MA). Tridecafluoro–1,1,2,2–tetrahydrooctyl–1–trichlorosilane was purchased from United Chemical Technologies, Inc. (Bristol, PA). FC–40 (a mixture of perfluoro–tri–n–butylamine and perfluoro–di–n–butylmethylamine) was obtained from 3M (St. Paul, MN). RfOEG (triethyleneglycol mono[1*H*,1*H*-perfluorooctyl]ether) was prepared according to published procedures.[1] The Access Ultrasensitive Insulin Assay obtained from Beckman Coulter contains capture antibody immobilized on paramagnetic beads, detection reporter antibody labeled with bovine alkaline phosphatase (ALP), blocking buffer, washing buffer, and standard insulin solutions S0 (blank), S1 (7.0 pM), S2 (70 pM), S3 (350 pM), S4 (1050 pM), S5 (2100 pM). The suspension of beads used in Figure 1 was a solution of mouse monoclonal anti-insulin coupled to paramagnetic particles, concentrated six times. Food dyes were purchased from Ateco (Glen Cove, NY). Three food dyes (orange, red, and blue) were diluted ~10 times from their stock solutions, and were filtered with a 0.45 μm PVDF syringe

filter before use. Grade N42 Neodymium (NdFeB) Magnets were purchased from K&J Magnetics (Jamison, PA).

Fabrication of the top plate of SlipChip with nanopatterning. A blank glass plate with photoresist coating (Soda-lime glass, thickness: 0.7 mm; chromium coating: 1025 Å; AZ photoresist: 1 μm) was first cut to be 2 in × 1 in. The glass plate with photoresist coating was aligned with a photomask containing the design for the wells and ducts. The photomask and plate were exposed to UV light for 1 min. Immediately after exposure, the photomask was removed from the glass plate and the glass plate was developed by immersing it in 0.1 mol/L NaOH solution for 1 min. Only the areas of the photoresist that were exposed to the UV light dissolved in the solution. The exposed underlying chromium layer was removed using a chromium etchant (a solution of 0.6:0.365 mol/L $HClO_4$ / $(NH_4)_2Ce(NO_3)_6$). The plate was thoroughly rinsed with Millipore water and dried with nitrogen gas, and the back of the glass plate was taped with PVC sealing tape (McMaster-Carr) to protect the back side of glass. The taped glass plate was then carefully immersed in a plastic container with a glass etching solution (1:0.5:0.75 mol/L $HF/NH_4F/HNO_3$) to etch the glass surface that was exposed after the chromium coating was removed. A 40 °C constant-temperature water bath shaker was used to control the etching speed. By control the etching time (~60 min), wells and ducts that were 80 μm deep were etched into the glass plate. The plate was thoroughly rinsed with Millipore water and dried with nitrogen gas. After ducts and wells were etched into the plate, the plate was aligned with a nanopatterning photomask and the same procedure was followed as above. After removing the chromium coating, the glass plate was immersed in 50:25:37.5 mmol/L $HF/NH_4F/HNO_3$ etching solution, and etched for 10 min at room temperature to produce ~300 nm deep patterns over the surface. Finally, the glass plate was rinsed with ethanol to strip the undeveloped photoresist, and

immersed in the chromium etchant to remove the chromium coating. The glass was then rinsed with ethanol and Millipore water and dried with nitrogen gas. The etched patterns were verified using a Veeco Dektak 150 profilometer. The glass plates were cleaned and subjected to an oxygen plasma treatment, and then the surfaces were rendered hydrophobic by silanization in a vacuum dessicator for 3 hours with Tridecafluoro−1,1,2,2−tetrahydrooctyl−1−trichlorosilane as previously described.[2] After silanization, the glass plates were baked in a 120 °C oven for 30 min, rinsed by immersing in a tank of FC-3283, and dried in a 60 °C oven overnight.

Fabrication of the bottom plate of SlipChip with hydrophilic wells. The glass plate was first prepared for etching using the same procedure as above. Before etching, both the area designed for the wells and the back of the glass plate were taped to be protected. Using an etching time of ~52.5 min, 70 μm deep ducts were etched on to the glass plate. After the plate was thoroughly rinsed with Millipore water and dried with nitrogen gas, the tape protecting the area designed for the wells was removed, and the plate was etched for ~7.5 min more. Wells that were 10 μm deep were etched into the glass plate, and the 70 μm deep ducts were further etched to a final depth of 80 μm. Then, the glass plate was rinsed with ethanol to strip the undeveloped photoresist. 1 mL of OmniCoat was evenly spread over the surface of the plate with a plastic pipette and spin-coated. The spin speed was set to be 3000 rpm/30 second. Once the coating was finished, the plate was baked on a 200 °C hotplate for 1 min and cooled to room temperature. Then, 20 μm SU8 2025 (Microchem) was spin-coated on the plate and pre-baked on a 95 °C hotplate for 3 min. The plate was flipped upside-down and covered with a photomask that protected the area containing the ducts. The plate was then exposed to UV light for 6 min. In the area exposed by the photomask, UV light only passed through the plate where the chromium coating was removed, so only the SU8 in the wells remained after developing. Next, the glass plate was

immersed in MF CD26 to develop the exposed OmniCoat, and then immersed in the chromium etchant to remove the chromium coating. The glass was then rinsed with ethanol and Millipore water and dried with nitrogen gas. The surfaces of the etched glass plates were cleaned and subjected to an oxygen plasma treatment, and then the surfaces were rendered hydrophobic by silanization in a vacuum desiccator as described above. The SU8 in the wells protected the wells and prevented them from being silanized. Finally, the glass plate was immersed in Remover PG at 80 °C for 30 min to strip the SU8 in the wells.

Operation of the SlipChip. Access holes were drilled with a diamond drill bit 0.030 inches in diameter. To assemble the SlipChip under FC-40, the top plate was placed onto the bottom plate. The ducts in the bottom plate were overlapped with the empty wells in the top plate, forming a continuous fluidic path for loading of the sample. The two halves of the device were secured by using two small binder clips. To load a solution into the SlipChip, a 10 μL pipettor tip containing a solution was inserted into the inlet of the fluidic path in the assembled SlipChip. The solution was dispensed into the ducts and wells by pushing the button of pipettor manually. To slip to mix a solution with another one, the top plate was moved relative to the bottom plate to align the wells in the top plate with the wells in the bottom plates.

Preparing and loading plugs for insulin assay in the SlipChip. Insulin solutions of 10 pM, 100 pM, and 1000 pM were made by diluting the standard solutions in washing buffer. Eight arrays of plugs, containing standard solutions of insulin S0, S2, S3, S4, S5, insulin solution of 10 pM, 100 pM, and 1000 pM, respectively, were prepared in a flow-focusing device. A 10 cm long piece of Teflon tubing (O.D. 225 μm, I.D. 150 μm) was inserted into the junction of the flow focusing device and was then sealed with wax to be air-tight. FC-40 was used as the carrier fluid. The flow rates for both the carrier fluid and the solutions were 1 μL/min. At these flow rates, the

volume of the plugs was ~ 5 nL. Once the 10 cm long piece of Teflon tubing was fully filled with plugs of a particular insulin solution, the flow was stopped. The Teflon tubing was then sealed at both ends with wax and stored in glass tubing (O.D. 3 mm, I.D. 2 mm) refilled with FC-70.

To load the SlipChip, the top plate of the SlipChip (500 mm thick), with the wells and ducts facing up, was placed in a 3.5-inch Petri dish containing 2–3 mm deep FC-40. A piece of Teflon tubing containing the plugs of an insulin solution was connected to a 5 cm-long piece of Teflon tubing (I.D. 370 μm), which was connected to a 10 mL syringe. Using a micro-manipulator to drive the syringe, the plugs were deposited into the designated wells on the Slip Chip, each plug into one well. The plugs of the eight solutions were deposited into the top plate of the SlipChip with the pattern shown in Figure 4. The plates were carefully manipulated so that the deposited droplets would not float away. The bottom plate was then laid on top of the top plate and the SlipChip was assembled. The procedure of the insulin assay in the SlipChip was the same that for calibration.

Data collection and analysis. Microphotographs were taken with a Leica MZ 16 Stereoscope (Leica Microsystems) with a Plan APO 0.63× objective. The fluorescence intensity was measured by using a Leica DMI6000 microscope (Leica Microsystems) with a 20 × 0.4NA Leica objective and a Hamamatsu ORCAER camera. A GFP filter was used to collect fluorescein fluorescence. Measurements were taken every 3 min. Images were acquired and analyzed by using Metamorph imaging system version 6.3r1 (Universal Imaging). First, the maximum intensity of the images was plotted against time, and then the initial increasing rates within ~ 30 min were extracted.

Insulin assay in the SlipChip by using back-and-forth slipping. The SlipChip used in assays with back-and-forth slipping was identical to one described previously.[3] Plugs of insulin solutions were prepared and loaded into the SlipChip as described above. See details of the step-by-step operation in the text and in Figure 5.

(1) Roach, L. S.; Song, H.; Ismagilov, R. F., *Anal. Chem.* **2005**, *77*, 785-796.
(2) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M., *Anal. Chem.* **1998**, *70*, 4974-4984.
(3) Du, W. B.; Li, L.; Nichols, K. P.; Ismagilov, R. F., *Lab Chip* **2009**, *9*, 2286-2292.

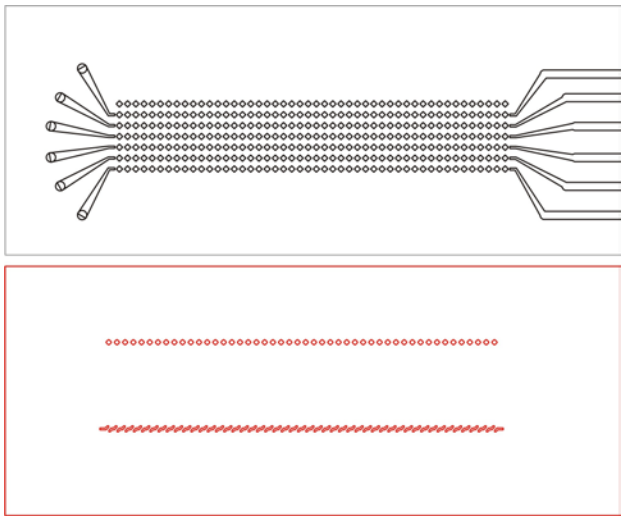

Figure S-1 The design of the SlipChip to analyze plugs. The assembled SlipChip is shown in Figure 4. Top panel: The schematic of the top plate of this SlipChip. This plate contained inlets, outlets, and wells for the various reagents (Section A), and wells for depositing plugs (Section B). All wells and ducts are 80 μm deep. Bottom panel: The schematic of the bottom plate of this SlipChip. This layer contained the 80 μm deep ducts to load the reagents (Section A), and the10 μm deep wells for the sample (Section B).