SUPPORTING INFORMATION

Nanoporous membranes enable concentration and transport in fully wet paper-based assays

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EXPERIMENTAL

Figure S1 shows the fabrication and assembly procedures for the external devices and are detailed as follows: (1) cut defined geometry from AutoCAD into silicone and PMMA using a CO_2 laser printer, (2) elevate the silicone onto rulers and pipette Nafion solution into the through-cuts, followed by baking for 30 min. at 65 ^{o}C, (3) place the silicone in a Petri dish and hydrate in buffer for 30 min., (3) assemble silicone and PMMA layers using bolts and nuts, and (4) place assembled device on a paper-based assay, fill buffer reservoirs and apply voltage.

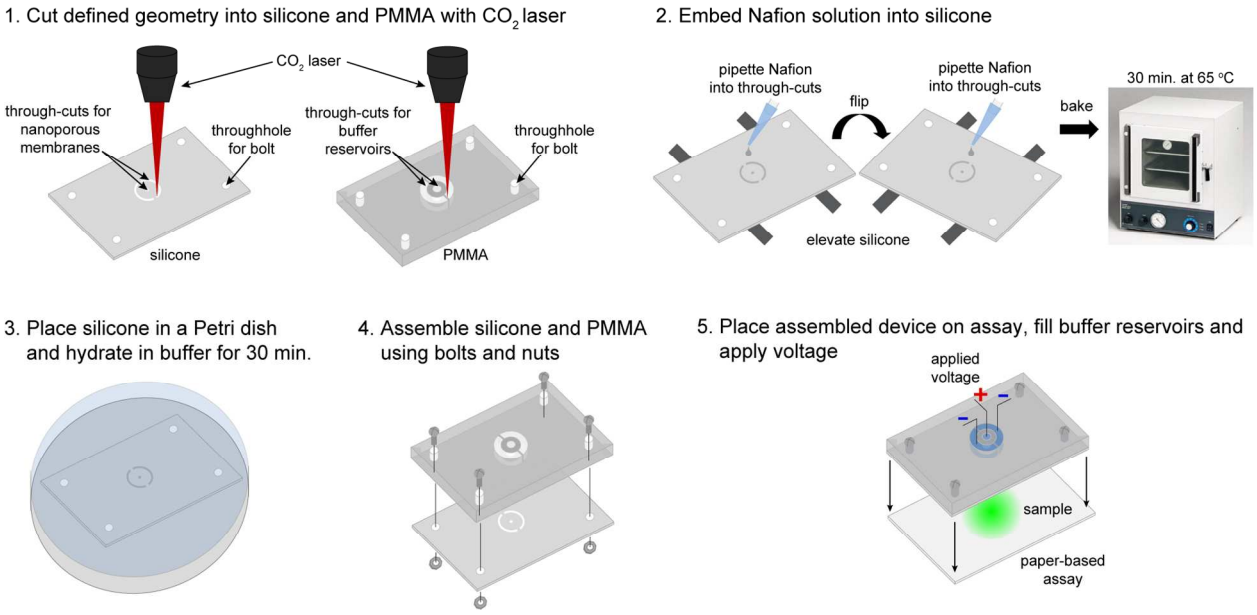

Figure S1. Fabrication and assembly procedures for the external devices.

RESULTS AND DISCUSSION

Figure S2 shows the results for concentration of fluorescent tracer in nitrocellulose membrane patterned with nanoporous membranes. Under similar experimental conditions used for Whatman No. 1 paper (*i.e.* applied voltage of 50 V), fluorescent tracer is concentrated within the same timeframe of 8.5 minutes. The peak concentration factor reaches a maximum value of 16-fold as compared to 22-fold for the No. 1 paper. The concentration process, however, is comparatively faster as the concentration factor plateaus earlier in the nitrocellulose membrane. This difference can be attributed to the more uniform pore structure in nitrocellulose membranes and consequently, more uniform transport rates.

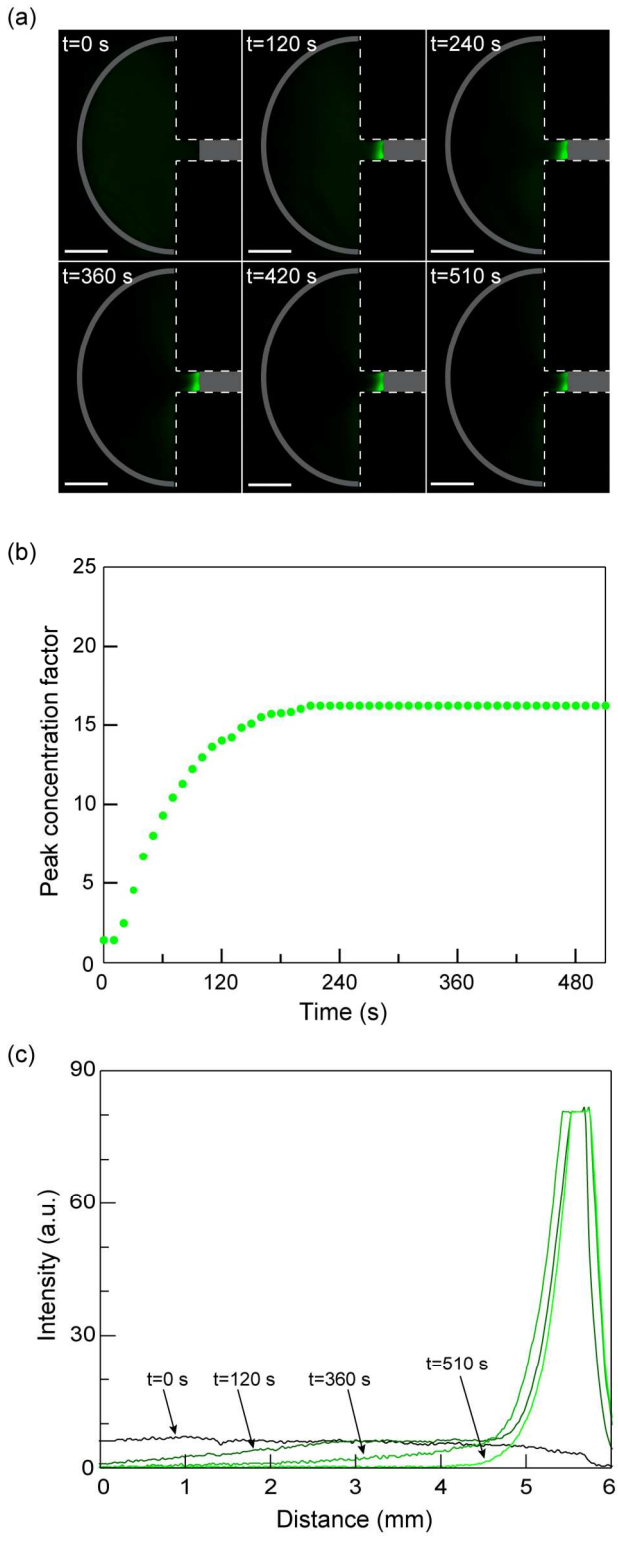

Figure S2. Active concentration in nitrocellulose patterned with nanoporous membranes. (a) Contrast enhanced images of fluorescent tracer concentration under an applied voltage of 50 V. Patterned inner and outer nanoporous membranes are indicated by the rectangle and arc, respectively. Patterned wax boundaries are indicated by the

dashed lines. (b) Time evolution of peak concentration factor. The maximum value reaches 16-fold at t = 210s. (c) Intensity profiles at selected time points, as indicated. Scale bars are 2 mm.

Figure S3 shows the results for the transport of fluorescent tracer using the in-paper device with a cover slip over the sample region. Fluid evaporation is minimized by the cover slip. By comparing the area under the curve (AUC) of the first and second cycles to the AUC of the initial concentration step, transport efficiencies of ~90% are quantified for both cycles. The total transport time for each cycle is 7.5 minutes which equates to a transport rate of 1.1 mm/min per cycle, over the ~8 mm distance.

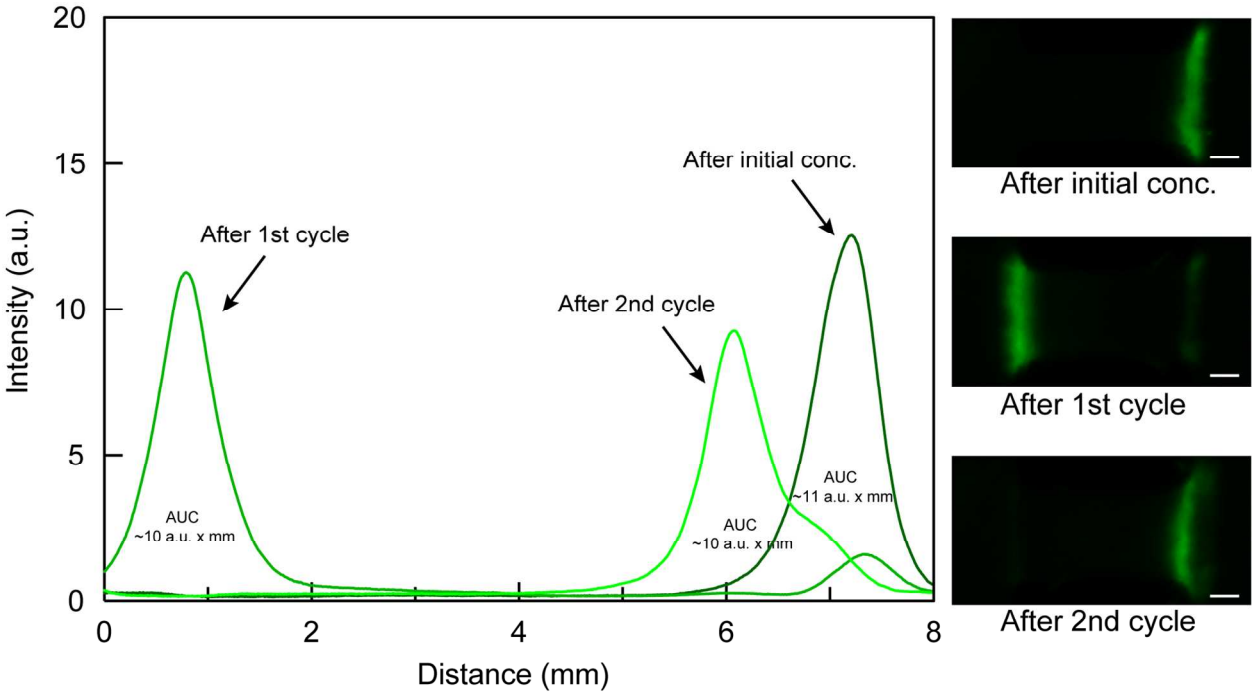

Figure S3. Active transport using the in-paper device with a glass cover slip over the sample region. The final intensity profiles are plotted for the initial concentration step, and first and second cycles. Contrast enhanced images are shown for the transport process. Scale bars represent 1 mm.

Table S1 details the material costs for the external and in-paper devices. The total cost per device for all device classes is <0.50 USD, with the nitrocellulose membrane device at the highest cost of ~0.41 USD.

Table S1. Material cost of external and in-paper devices.

Material	**Supplier**	**Product No.**	**Unit**	**Total cost (USD)**	**Unit cost (USD/cm^2)**	**Device cost (USD)**		
						External	**CHR**	**NC**
No. 1 chromatography paper (CHR)	Sigma Aldrich	WHA3001861	400 cm^2/sheet 100 sheets/pk	55.40	0.001385	N/A	0.006648	N/A
Nitrocellulose (NC) membrane	Sigma Aldrich	Z6709091	400 cm^2/sheet 5 sheets/pk	160.60	0.0803	N/A	N/A	0.38544
Nafion 117 solution	Sigma Aldrich	70160	25 mL	131.50	0.00526 (USD/µL)	0.1052	0.01578	0.01578
Solid ink, black	Xerox	108R00726	30 cm^2/sheet ~1100 sheets/stick	84.99	0.002575	N/A	0.005562	0.005562
Silicone rubber sheet	McMaster Carr	5827T21	900 cm^2/sheet	14.77	0.016411	0.221549	N/A	N/A
Acrylic sheet (PMMA)	Plastic World	AC3MM4x8	900 cm^2/sheet	1.91	0.002122	0.028647	N/A	N/A
				Total cost per device:		*0.355396*	*0.02799*	*0.406782*

Table S2 details the fabrication time required for the external and in-paper devices. The in-paper devices can be fabricated in <60 min. and the external device in 74 min.

Table S2. Fabrication time required for external and in-paper devices.

Task	**Time required (min)**		
	External	**CHR**	**NC**
Wax printing	N/A	0.5	0.5
Wax melting	N/A	2	5
Nafion patterning	3	1	1
Nafion baking	30	15	15
Nafion hydration	30	30	30
Silicone micromachining	5	N/A	N/A
PMMA micromachining	5	N/A	N/A
Silicone to PMMA assembly	1	N/A	N/A
Total time required per device:	*74*	*48.5*	*51.5*

N.B.: Wax printing and melting, and Nafion baking and hydration can be done in parallel for up to 40 devices for the 20 cm x 20 cm No. 1 paper and NC membrane sheets used.

List of Movies:

1. Active concentration of fluorescent tracer using the external concentration device at 12x speed. The inner and outer membranes are overlaid as the dot and dashed circle, respectively.
2. Active transport of fluorescent tracer using the external transport device at 56x speed. Membranes are overlaid as the dashed lines.
3. Active concentration of fluorescent tracer using the in-paper concentration device at 30x speed. The patterned membranes are indicated by the solid arc and rectangle. Wax boundaries are indicated by the dashed lines.
4. Active transport of fluorescent tracer using the in-paper transport device at 58x speed. The patterned membranes are indicated by the solid bands.

All images in the movies were contrast enhanced prior to conversion into videos.