Supporting Information

Integrated Poly(dimethysiloxane) with an Intrinsic Nonfouling Property Approaching "Absolute" Zero Background in Immunoassays

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Materials and Methods

The vinyl terminated initiator (10-undecen-1-yl 2-bromo-2-methylpropionate) was obtained from HZDW (Hangzhou, China). $CuCl_2$, 2, 2'-bipyridine, sodium hydroxide and bromoacetic acid were purchased from Acros. Oligo(ethylene glycol) methacrylate (OEGMA, M_n=526), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Aldrich. Monomers were used as received. All the antibodies, antigens, and reagents for the multiplexed ELISA assay were received from HealthDigit (Shanghi, China) as gifts. Serum samples were collected from local hospitals with consent from the patients.

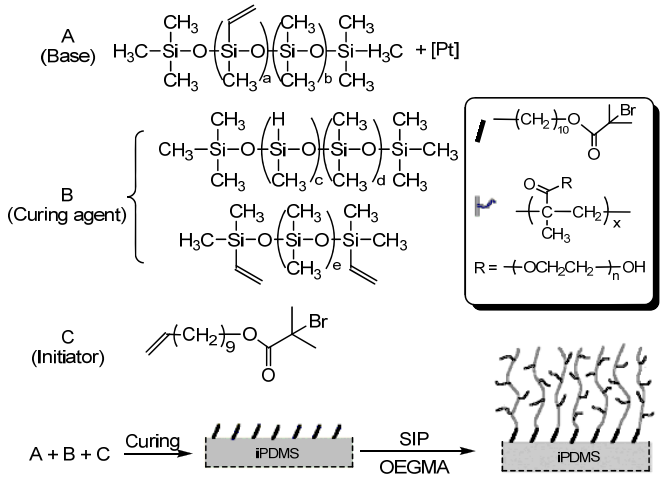
Buffer/other solutions used and their abbreviations: *Phosphate buffer* (PB, 0.3 M, pH~7.4); *Phosphate buffered saline* (PBS, 0.1 M, pH~7.4); *Phosphate buffered saline-Tween 20* (PBST, 0.01 M, add 0.02% (w/v) Tween 20 to PBS, pH~7.4); *Tris-buffered saline* (TBS, 0.1M, pH~7.6); *Tween-Tris-buffered saline* (TTBS, 0.1 M. add 0.02% (w/v) Tween 20 to TBS, pH~7.6); *EDC/NHS activation solution* (an aqueous

solution contains 0.1 M (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and 0.1 M (N-hydroxysuccinimide)); *Blocking solution* (add 5% BSA (w/v) and 4% Sucrose (w/v) to PB); *Probing solution* (add mixture of antigens from Table 1 or unknown samples, 5% BSA (w/v) and 2% Sucrose (w/v) to PB); *Array solution* (add one Capture antibody from Table 1, 0.01% Triton x-100 (w/v), 0.2% Glycerol (w/v) and 1.5% Mannitol (w/v) to PB); *Detection solution* (detection antibodies from Table 1 were diluted with guardian peroxidase conjugate stabilizer/diluents). The final concentrations of capture and detection antibodies were listed in Table 1 unless otherwise stated.

Preparation of iPDMS sheets: To prepare iPDMS, the third component C (Scheme S1), was first mixed well with PDMS (Sylgard 184) precursor (A) and curing agent (B) at a ratio of 10:1:0.1 (A:B:C), which could react with hydrosilane hydrogens in the presence of Pt catalyst. This mixture was then poured into a 6.6 × 7.7 cm^2 mold and cured at 80 °C for 2 hours, resulting in a transparent elastomer.

The resulted iPDMS was then subjected to surface modification via surface initiated polymerization (SIP) as previously described[1]. The SIP reaction mixture had a mole ratio of OEGMA526/$CuCl_2$/Bipy/AscA =100/1/2/1, with a feed [$CuCl_2$] at 2.76 mM. SIP was conducted under argon protection and continued for 120 min at ~25 °C. Polymerization was stopped when iPDMS was removed from the solution. Samples were thoroughly rinsed with methanol, Milli-Q water, and dried under flowing nitrogen.

The now poly(OEGMA) coated iPDMS sheets were further functionalized by incubating in an aqueous solution of 1 M bromoacetic acid and 2 M sodium hydroxide for overnight to generate terminal carboxyl groups, followed by washing with milli-Q water for three times, dried under flowing nitrogen and stored at 4 °C for future use.



Scheme S1. Preparation of iPDMS and surface modification of iPDMS via surface initiated polymerization (SIP) of OEGMA.

Protein Microarray (PM): The polymer coated, COOH functionalized iPDMS (referred to as iPDMS thereafter) was first immersed into an aqueous solution containing 0.1 M EDC and 0.1 M NHS for 30 min. The sheets were washed with Milli-Q water for three times, dried under flowing nitrogen and used immediately. A HD-2003A noncontact plotter (Figure S1) was utilized to array capture antibodies (i.e.,

array solutions) on either NC or iPDMS membranes at 25 °C and 50% humidity. The configurations of PMs as well as the contents of array solutions, probing solutions and detection solutions were dependent on the experimental needs and they were presented along with data analysis.

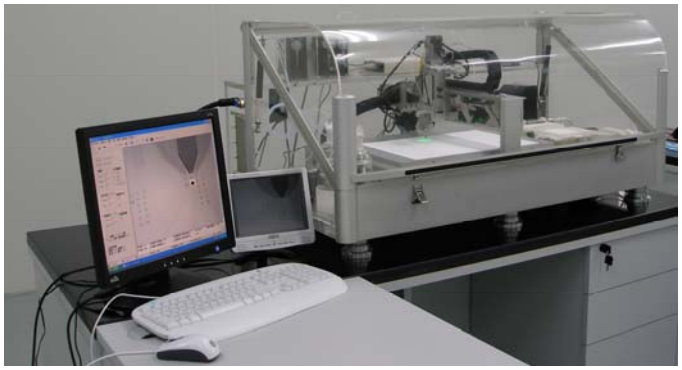


Figure S1. A HD-2003A plotter (HealthDigit, Shanghai, China) shown on the right was used for PM fabrication. The green spot indicated where the proteins were jet-printed, which were displayed on two screens in real-time.

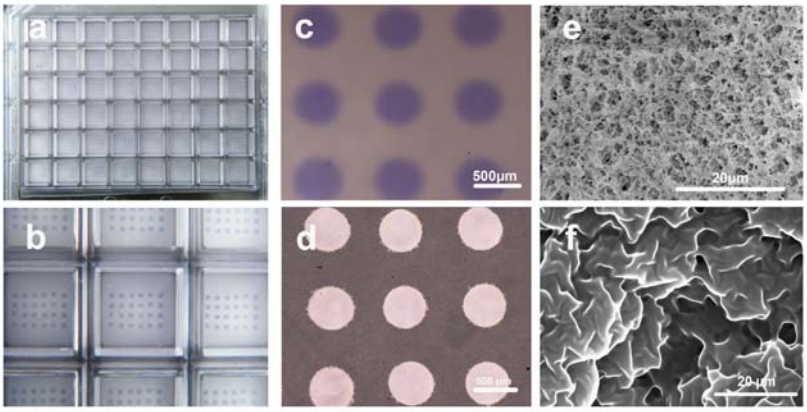


Figure S2. PM under digital camera, light microscope and SEM. (a) digital camera image of vertical view of a completed 48-well PM on iPDMS sheet, (b) zoomed to show the 24 dots. Each spot consumed 10 nL of printing solution on iPDMS and 20 nL on NC substrate. Nine spots on a NC substrate (c) and iPDMS (d), respectively, were imaged under white-light microscope. The resulting spots were typically with 500 μm diameter and a pitch of 400 μm between spots. The shape indicated high quality of printing process. For (c), the tiny black spot at the lower right corner was on the surface of the NC film and was used as a focus reference point. The blurred nature of the spots was due to the fact that NC was a three dimensional, porous material. Protein solutions were absorbed instead of staying on the top of surface (i.e., absorption vs. adsorption). The SEM images were shown for NC substrate (e), and iPDMS (f), respectively. The morphology of polymer coated iPDMS has pseudo-3D structure (1~2 μm in the Z direction), which leads to a higher specific surface area when compared with 100 nm poly(OEGMA) coated glass slides.

Microarray was prepared in a clean room (1000 grade). For iPDMS, it was first immersed into EDC/NHS activation solution for 30 min, followed by three times of Milli-Q water rinse, dried thoroughly under flowing nitrogen and used immediately. A HD-2003A noncontact plotter (HealthDigit, Shanghai) was utilized to array capture antibodies on either NC or iPDMS membranes at 25 °C and 50% humidity. The 6.6 × 7.7 cm^2 membrane surface was pre-divided into 48 squares (~ 8 × 8 mm^2 each). And to

each square, up to 11 different types of Array solutions (Table 1) were spotted in duplicates, 10 nL for each spot. After printing, iPDMS sheets were stored at room temperature for overnight. They were then placed on the base plate and covered with a cover plate, which was pressed to form a 48-well plate (Figure 1b). This complete cassette was then sealed under nitrogen protection for future use.

For NC film, there was no activation step so Array solutions were directly printed. After dry, NC film was assembled into a complete cassette. Different from iPDMS, the base plate and cover plate for NC were pre-inked with mucilage before pressing fit to avoid side leakage. The surface of NC film was further blocked with 0.1 mL blocking buffer for 1 h, washed with PBS. After dried for overnight, NC cassette was then sealed under nitrogen protection for future use. The shelf-life for NC and iPDMS microarrays was minimal six-month.

The content of the 24 dots in each well and the 48 wells of one iPDMS/NC sheet might be adjusted according experimental needs (Figure S3). For example, sometimes we printed additionally 2 spots of control (PB) thus each well contained 24 spots.

ELISA processing protocol: To a freshly opened iPDMS cassette, one added 0.1 mL of $NaHCO_3$ (0.1M) to each well, incubated for 5 min to achieve re-hydration. After rinsing with TTBS for 3 times, probing solutions were added and incubated at 37 °C for 30 min. Then the wells were immersed with TTBS for 8 min and rinsed. Detection solution was then added and incubated at 37 °C for 30 min., followed by 8 min of TTBS washing. After removal of the cover plate, chemiluminescence substrates (SuperSignal ELISA Femto Stable Peroxide Solution and SuperSignal ELISA Femto Luminol/Enhancer Solution from Thermo) were uniformly added on to the surface. Images were taken by a HD-2001 Chip Reader (HealthDigit, Shanghai, China) and LAS-4000 imaging system (Fujifilm, Japan). Data were analyzed by Bioca (Version 5.0, HealthDigit).

For NC, the protocol was similar with the one for iPDMS except that after each incubation step, the wells were immersed in TTBS for 4 × 8 min and rinsed thoroughly to reduce nonspecific protein adsorption.

The contents of probing solutions and detection solutions might be adjusted according experimental needs (Figure S3, S5, S6,S7, S11 and S13).

Data analysis: A signal is defined by equation (1), where $Intensity_{dot}$ and $Intensity_{non\text{-}dot}$ are readout of Bioca software:

$$S = Intensity_{dot} - Intensity_{non-dot} \quad (1)$$

Two parameters are important in evaluating the performance of PM, namely, coefficient of variation (CV) and limit of detection (LOD). CV is defined as equation (2), which is commonly used to evaluate the precision relative to the mean value.

$$CV = \frac{\mathrm{STD}}{Average} \times 100\% \quad (2)$$

LOD is defined as equation (3). It is the concentration that can produce a signal equivalent to S_{LOD}. For 99% of confidence, the value of S_{LOD} is S_{blank} plus 3 times standard deviation of blank value (σ_{blank}). S_{blank} is the readout when the probing solution contains no antigen (i.e., PB with 5% BSA and 2% Sucrose).

$$S_{LOD} = S_{blank} + 3\sigma_{blank} \quad (3)$$

Data was fitted according to a four-parameter logistic (4-PL) function by the following equation (4):

$$g(\log Y) = f(x,a,b,c,d) = a + \frac{b-a}{1+10^{(\log c - \log x)\times d}} \quad (4)$$

where x and Y are the dose and response, respectively, a, b, c, and d are the four parameters of the 4-PL function. The fit was performed by GraphPad Prism (Version 5.0).

Scan Electron Microscopy: Scanning electron microscope (HITACHI S-4800, Japan) was used to characterize iPDMS surfaces. The microscope was operated at 10.0 kV. Prior to measurements, the sample was coated with a thin gold film by means of a vacuum sputter to improve electrical conductivity.

Results and Discussion

The configurations of PMs were dependent on experimental needs. For example, Figure S3 was designed to test if the iPDMS could meet the requirements of clinical use, including CV, calibration curves and sample testing. This iPDMS was divided into three zones and all three zones were printed with 11 different array solutions (11 capture antibodies in Table 1, two dots for each tumor marker except that CK19 has four dots), resulting in 24 dots per well. Zone A has 10 wells, which were challenged with a probe solution that has 11 antigens. The concentrations of component antigens were 1/2 of what listed in Table S1 (denoted as C_3). Each well was then treated with a detection solution, which is a cocktail of 11 horseradish peroxidase conjugated detection antibodies (abbreviated as second Ab-HRP thereafter, Table S10). The results of this quality control were listed in Table S2: most of the tumor markers have a less 10% value of CV indicated that this PM on iPDMS met the clinical requirement (CV < 15%).

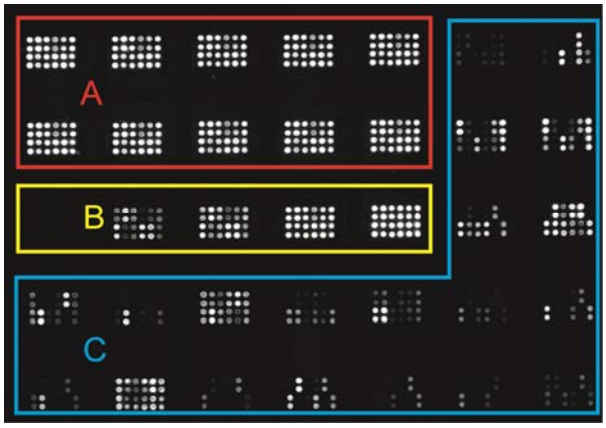


Figure S3. Design of PM on iPDMS depends on experimental needs. In this experiment, 13 wells were left out. The other 35 wells were divided into 3 zones, namely, (A) quality control for CV test (Table S2), (B) the internal calibration curves, which provides a simplified 5 dots fitting curve for quantification (Table S1 and Figure S4); (C) tests of patients' serum (Table S4).

Zone B has 5 wells that were used to construct internal calibration curves. From left to right, each well was challenged with probe solutions with increased concentrations of antigens. Let the probing solution with antigens concentrations listed in Table S1 as C_4, C_3, C_2, C_1 and C_0 were 1/2, 1/4, 1/8 and 0 concentrations of C_4, respectively. These 5

wells were finally incubated with optimal detection solutions (Table S10), which provided a simplified 5 dots fitting curve (Figure S4) for quantification of unknown samples (i.e., patients' serum).

Table S1. Concentrations of component antigens in a cocktail (probing solution) used for dose-response calibration curves (denoted as C_4).

	AFP [a]	CEA [a]	c-PSA [a]	CK19 [a]	NSE [a]	SCC [a]	CA 15-3 [b]	CA 125 [b]	CA 19-9 [b]	CA 242 [b]	β-HCG [c]
C_4	150.7	65.3	42.9	30.9	68.6	28.1	67.7	298.1	198.5	113	30.2

[a] the unit is ng/mL; [b] the unit is U/mL; [c] the unit is mIu/mL.

Table S2. Coefficient of variation (CV) of multiplexed ELISA from intra-assay. (Data from zone A of Figure S3, n = 20).

CV%	AFP	CEA	CK19	c-PSA	NSE	SCC	CA125	CA 19-9	CA15-3	CA 242	β-HCG
NC	7.9	9.6	7.4	5.2	5.1	7.1	5.6	10.8	5.0	9.3	5.1
iPDMS	6.0	10.6	7.3	5.5	9.5	2.7	5.1	6.3	6.2	3.6	6.0

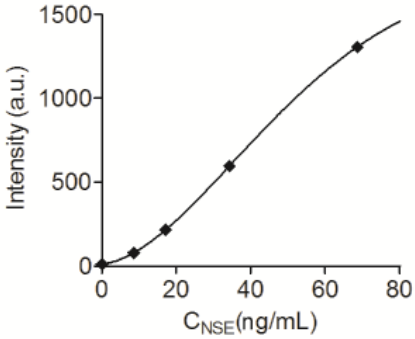


Figure S4. A representative internal calibration curve for NSE was constructed from the data of Figure S3.

Zone C has 20 wells that were used to test serum samples from patients (Table S4). The values of $C_{hospital}$ were obtained by routine single indexed immunoassay and were used for reference purpose only (i.e., an indication of "positive" sample). The values of C_{iPDMS} and C_{NC} were obtained via parallel PM experiments on iPDMS and NC thus they could be compared quantitatively. For 37 tested samples (from multiple iPDMS sheets), all were confirmed to be positive although the absolute values were different. We are conducting large scale experiments and will report them in the future. We further determined the inter-assay CV value (Table S3): 6 tumor markers common for 20 iPDMS and NC sheets were selected. These samples were processed according standard protocol at different time but at the same conditions: incubated with corresponding antigens at C_3 concentration and detection antibodies of the same concentration. The inter-assay CV also indicated PM on iPDMS satisfied the clinical use.

Table S3. Inter-assay coefficient of variation (CV) of multiplexed ELISA (n = 20).

CV%	AFP	CEA	CK19	SCC	CA125	CA 19-9
iPDMS	9.4	10.6	7.2	8.5	7.7	13.9
NC	9.8	10.0	13.3	8.8	12.0	12.8

Table S4. Test iPDMS and NC with positive serum samples.

No.	Marker	$C_{Reference}$	$C_{hospital}$	C_{iPDMS}	C_{NC}
1	AFP (ng/mL)	<20	58.7	102.9	86.6
2			44.5	78.7	60.4
3			97.0	41.7	59.7
4			114.5	70.8	107.6
5			43.0	24.1	23.0
6			74.0	72.5	89.5
7	CA 125 (U/ml)	<35	82.8	62.1	52.7
8			89.1	43.3	48.0
9			99.9	51.2	65.7
10			83.2	61.1	59.4
11			243.1	208.4	201.3
12			332.0	124.4	212.9
13			124.5	38.7	64.9
14	CA 15-3 (U/ml)	<35	115.6	98.3	101.4
15			65.9	167.5	102.1
16			97.1	77.6	181.0
17			102.0	76.7	137.0
18	CA 19-9 (U/ml)	<35	98.7	114.6	94.1
19			95.0	80.5	78.3
20			43.7	68.7	34.4
21			314.4	57.7	58.5
22			751.8	165.7	107.9
23			584.1	156.9	77.3
24	CEA (ng/mL)	<5	19.2	9.5	9.0
25			28.1	>50	37.4
26			19.2	20.8	8.3
27			29.4	17.1	14.2
28			19.4	15.6	11.3
29			28.2	49.2	21.6
30			64.7	39.0	26.7
31			69.6	24.6	17.4
32			78.1	29.8	25.8
33	CK19 (ng/mL)	<3.3	11.4	8.0	7.8
34			75.5	8.4	19.0
35	NSE (ng/mL)	<13	110.2	15.3	14.1
36	SCC (ng/mL)	<1.9	16.7	3.3	5.0
37			7.5	4.1	3.9

LOD of 11 tested tumor markers.

To test the LOD of multiplexed ELISAs on iPDMS and NC, we printed 11 array solutions as listed in Table 1 and 1 PB solution as control. The probing solutions were cocktails of 11 antigens/tumor markers, typically 16 cocktails that were diluted from C_4 by a factor of 2, from 2 C_4 to 1/2048 C_4. A detection solution with 11 second Ab-HRPs as listed in Table S10 was used. The total concentration was at 2.15 μg/mL for NC and 10.24 μg/mL for iPDMS, respectively. A higher concentration of second Ab-HRP was used for iPDMS because iPDMS was nonfouling and able to maintain a low background even under high concentration of second Ab-HRP (See text below for detailed discussion).

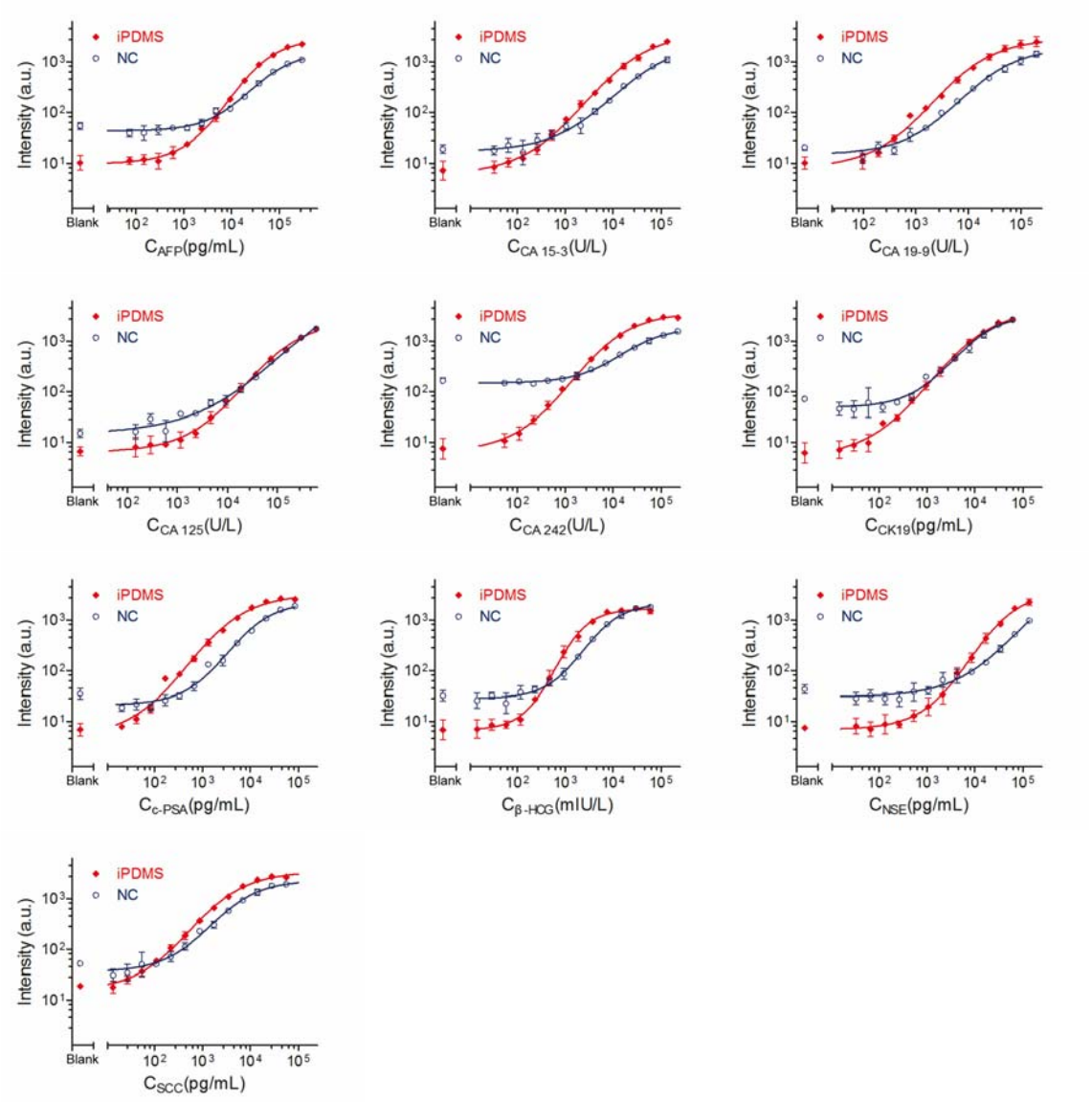


Figure S5. Dose-response curves of 11 different tumor markers (see CEA in text). The Array solutions were listed in Table 1, the probing solutions were series of 11 antigens diluted by a factor of 2, from 2 C_4 to 1/2048 C_4 (Table S1), the detection solution was a cocktail of 11 detection antibodies as listed in Table S10. The data was fitted using a 4 parameter logistic curve-fitting (y = bottom + (top - bottom)/{ 1 + 10^[(logEC50 - x) × HillSlope]}, (Prism 5.0, GraphPad Software, Inc.) and LODs were calculated for 99% of confidence (see LODs in Table 1).

Compare single indexed ELISA on iPDMS and NC.

Multiplexed ELISA and single indexed ELISA are different in several ways. To fabricate single indexed ELISA, we applied the same array solutions: capture antibodies of CEA, CA242 and c-PSA as listed in Table S5. Instead of printing 11 tumor markers in one well, we printed only one tumor marker in one well for single indexed ELISA. The probing solutions were also different: while multiplexed ELISA used a cocktail of 11 tumor antigens, the probing solution of single indexed ELISA contained only one antigen. For example, probing solution for the well with CEA capture antibodies contains only CEA. The most significant difference for multiplexed ELISA and single indexed ELISA came from the detection solution: while multiplexed ELISA used a cocktail of 11 detection antibodies as listed in Table 1 (total concentration at 2.15 μg/mL for NC), single indexed ELISA only used detection solution with one corresponding detection antibody at a much lower concentration. For example, the concentration of detection antibody for CEA was only 0.22 μg/mL for single indexed ELISA on NC. This concentration difference will greatly change the background intensity due to NPA of detection antibodies therefore the performance of PM on NC. Such influence was not seen on iPDMS (Figure S6 and Table S6). Moreover, iPDMS showed a better Signal-to-noise ratio than NC at all concentrations of targeting protein (Figure S7).

Table S5. The experimental parameter of single indexed ELISA.

		CA 242	CEA	c-PSA
iPDMS	**Array solution**	0.04 mg/mL	0.08 mg/mL	0.1 mg/mL
	Probing solution [a]	0~3200 U/mL	0~400 ng/mL	0~640 ng/mL
	Detection solution [b]	0.16 μg/mL	0.11 μg/mL	0.5 μg/mL
NC	**Array solution**	1 mg/mL	0.6 mg/mL	1 mg/mL
	Probing solution [a]	0~3200 U/mL	0~400 ng/mL	0~640 ng/mL
	Detection solution [b]	0.07 μg/mL	0.05 μg/mL	0.35 μg/mL

[a] Table shows the concentration range of antigen tested, probe solutions were serially diluted by a factor of 2, [b] Instead of using a cocktail of 11 detection antibodies, only one corresponding detection antibody was used.

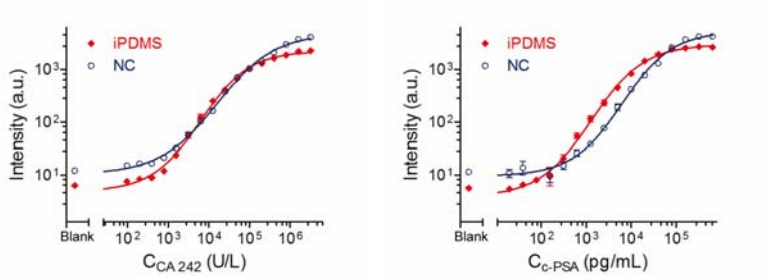


Figure S6. Representative dose-response curves of single indexed ELISA on iPDMS and NC. The big difference between iPDMS and NC on the value of LOD diminished because BSA blocking was effective for low concentration of detection antibodies (Table S6)

Table S6. LOD of single indexed vs. multiplexed ELISAs on iPDMS and NC.

	CA 242 (U/L)		CEA (pg/mL)		c-PSA (pg/mL)	
	Multiple	**Single**	**Multiple**	**Single**	**Multiple**	**Single**
LOD_{iPDMS} *	105	177	109	137	39	53
LOD_{NC}	2234	178	552	132	825	169

* Higher concentration of detection antibodies were used in multiplexed ELISAs in than single ones, then results in a better performance of LODs on iPDMS.

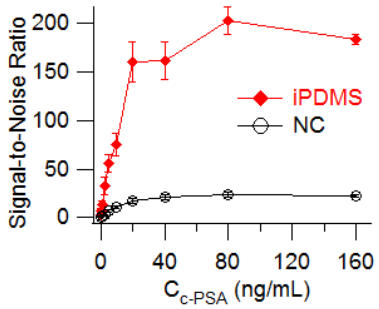


Figure S7 Comparison of signal-to-noise ratios between iPDMS and NC at different target protein concentrations (take c-PSA as example). The signal-to-noise ratio was calculated as the ratio between the mean spot signal and the mean of NPA noise around the pattern area (total noise subtracting instrument noise, Average ± STD, n = 12).

We also compared the signal-to-noise ratios from other microarrays based on sandwich procedure (Table S7). Note that such comparison should be taken with caution because different calculation methods and standards were used in different literatures.

Table S7. The maximal signal-to-noise ratios of protein microarrays on different substrates.

Detection method	Signal-to-noise ratio	Substrate
fluorescence	~9	Modified glass slides[2]
	183±48	Silicon grafted slides[3]
	43±20	Glass slides[3]
	up to 57	Commercial glass slides[4]
	~1050	Modified NC[5]
	up to 770	Modified glass slides[6]
	~40	silicon substrates[7]
	>100	PEG coated slides[8]
	250	PEG coated slides[9]
chemiluminesence	600	PEG coated slides[10]
	~20	NC[a]
	~200	p(OEGMA) coated iPDMS[a]

[a] estimated from the single indexed immunoassay of c-PSA.

Near "Absolute" Zero Background for iPDMS.

The instrument noise was given by taking an image without iPDMS or NC, which was mainly attributed to thermo noise of cooled CCD (Table S8). To determine the background signal from NPA of second Ab-HRP on iPDMS and NC, we first added detection solutions at different concentrations into the wells pre-incubated in probe solution free of any antigens (i.e., C_0), followed by second Ab-HRP incubation. After washing, chemiluminescence substrates were uniformly added on to the surface and images were taken as routine. The background readings (i.e., noises on iPDMS and NC) were a combination of instrument noise and the noise from nonspecifically adsorbed second Ab-HRP. The iPDMS shown an extremely low background compared to NC (~4 for iPDMS vs. ~98 for NC after subtracting 58 of the instrument noise) when the concentration of second Ab-HRP was ~2 μg/mL. Such difference was obvious from

Figure S8. Given its superior nonfouling property, we were able to use higher concentrations of second Ab-HRP to achieve a lower LOD, similar to the use of a higher power amplification method. For example, we tested second Ab-HRP concentrations as higher as 10.24 μg/mL and background reading was still negligible (~10). There is a key advantage from this intrinsically low background: iPDMS will be more reliable when detecting a low concentration sample, i.e., Signal ~20 with a background ~ 9 for iPDMS vs. the same signal but a background ~ 98 for NC.

A time-resolved background experiment was also presented (Figure S9). The NC film was pre-blocked with blocking buffer for 1 hour, then both NC and iPDMS were cultured in 100 ng/mL second Ab-HRP. NC film showed an obvious time-resolved non-specific adsorption rising, while iPDMS showed a constant low background. Note that data was collect by LAS-4000 imaging system (Fujifilm, Japan).

Table S8: The average background signal level at different situations (Average ± STD, n = 72).

	Instrument noise	**iPDMS**				**NC**	
$C_{second\ ab}$(μg/mL)	0	0	2.6	5.3	10.6	0	2.0
Signal (a.u.)	58±1	58 ± 1	62 ± 1	67 ± 2	70 ± 4	65 ± 1	156 ± 40

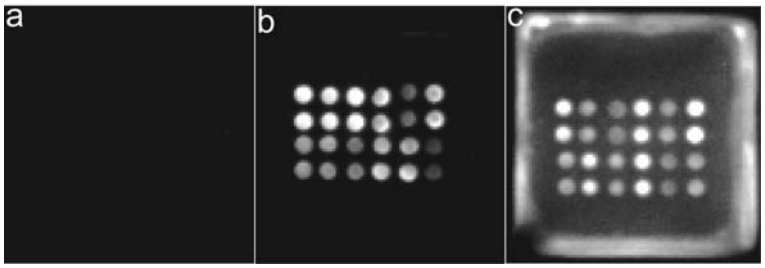


Figure S8. Local view of different array areas without grayscale adjustment: (a) iPDMS without any antigens or detection antibodies; (b) iPDMS with antigen C_3 and detection antibodies (total concentration at 2.26 μg/mL); (c) NC with antigen C_3 and detection antibodies (total concentration at 2.15 μg/mL). The bright "ring" was due to the glue applied to seal the well. The intensity at non-doted area was also increased due to the failure of surface chemistry.

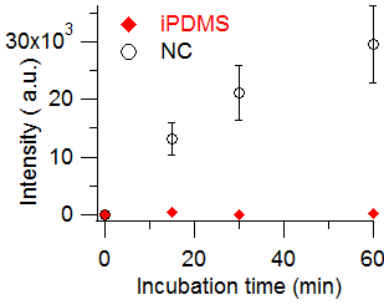


Figure S9. A time-resolved comparison of non-specific adsorption of second Ab-HRP was made between NC and iPDMS. Reported values of intensity were Average ± STD, n = 48.

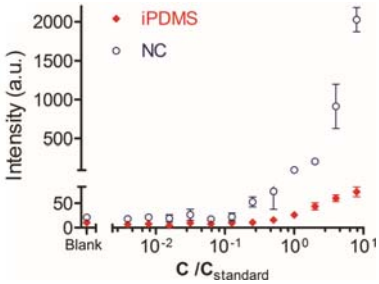
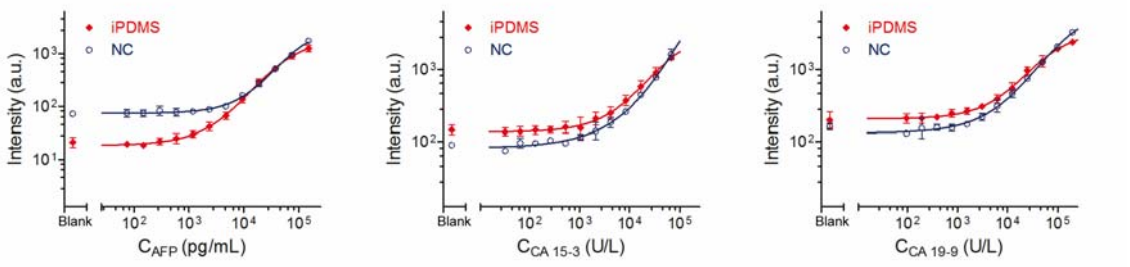


Figure S10. Multiplexed ELISA required a solid supporting material with superior nonfouling property. Standard printing and ELISA assay protocols were applied except (1) probing solutions contained all other 10 tumor markers (see Table S1) except CA 15-3, (2) the detection solution contained all other 10 second Ab-HRPs except for CA 15-3. C_{std} was the total concentration of the 10 tumor markers at C_4 listed in Table S1 and C/C_{std} was used because of the different concentration units for different tumor markers. iPDMS (red dots) showed a below instrument detection limit of noise when C/C_{std} was less than 1. The rising of intensity (up to 74) for C/C_{std} >1 were attributed to the mismatch between the capture antibody of CA 15-3 and other CA series antigens. This level of background was acceptable for most assays. For NC, however, the background reading at C/C_{std} = 8 was 2025, which was ~30-fold higher compared with the 74 reading for iPDMS. Assuming the mismatch of antigen-antibody pairs caused the same level of background (i.e., 74), NPA of tumor markers and second Ab-HRP contributed 1950 background intensity, which was almost half of the dynamic range of CCD readings (60-4000 a.u.). This control experiment clearly demonstrated the importance of an "absolute" zero background.

Negative serum is not "Negative".

In this set of experiments, both array solutions and detection solutions were kept the same as listed in Table 1. We replaced PB with "negative serum" in probing solution. The intensity reading of background for iPDMS and NC were 65 ± 8 and 186 ± 47 (n = 16). The low background of iPDMS was attributed to its intrinsic nonfouling property. The "blank signal" (probed with C_0) on iPDMS and NC increased from 67 ± 2 to 139 ± 68 (n = 72) and from 156 ± 40 to 240 ± 48 (n = 72), respectively. Compared with PB, "negative serum" caused a ~80 increase of "blank signal" for both iPDMS and NC, implying the "negative serum" was not negative (Table S9). These could be explained by the fact that there are always trace amount of tumor markers in healthy person (the donor for "negative" serum), some concentration of which were already beyond the LOD of iPDMS and NC. Since iPDMS could give a higher signal-to-noise ratio at low concentration than NC, a more reliable value could be given by iPDMS rather than "negative" result given by NC or traditional ELISA in clinical diagnosis of healthy person, if necessary.



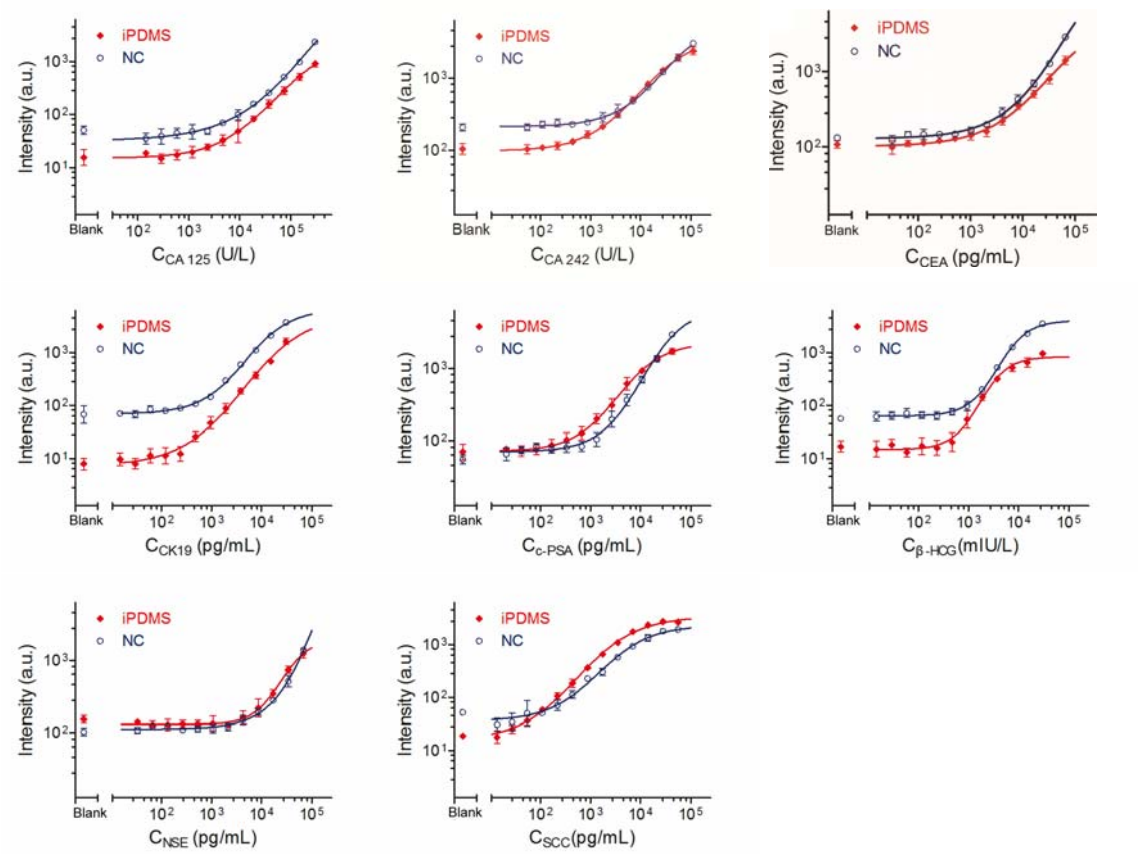


Figure S11. Dose-response curves of 11 different tumor markers on iPDMS and NC from multiplexed ELISAs. The antigens were diluted in "negative" serum (i.e., PB was replaced with "negative" serum in Probing solution).

Table S9. List of LOD from multiplexed ELISAs that used "negative" serum to replace PB in probing solutions.

	AFP (pg/mL)	CEA (pg/mL)	CK19 (pg/mL)	C-PSA (pg/mL)	NSE (pg/mL)	SCC (pg/mL)	CA 15-3 (U/L)	CA125 (U/L)	CA 19-9 (U/L)	CA 242 (U/L)	β-HCG (mIU/L)
LOD_{iPDMS}	1245	757	124	438	7430	39	1950	3342	3311	617	500
LOD_{NC}	2404	740	883	279	3524	134	760	2985	2547	1585	434
R_{iPDMS}[a]	1.8	6.9	1.9	11.2	39.7	2.1	13.9	6.7	18.9	5.9	7.2
R_{NC}[b]	0.6	1.3	1.9	0.3	0.9	0.8	1.8	5.0	4.4	0.7	0.9
R_{serum}[c]	1.9	1.0	7.1	0.6	0.5	3.4	0.4	0.9	0.8	2.6	0.5

[a] The ratio between the LOD of different tumor makers diluted in "negative" serum and in buffer, on iPDMS; [b] the same ratio as [a] except on NC; [c] the ratio between the LOD of different tumor makers on NC and iPDMS, with the antigens being diluted in "negative" serum.

Connections between the LOD and dissociation constant (K_D).

Surface Plasmon resonance (SPR) was used to determine the dissociation constant of affinity pairs. SPR chip were coated with a COOH functionalized poly(OEGMA) brush.

We conducted SPR experiments in a sequence that was the same as of the ELISA: capture antibodies was first immobilized to SPR chip, followed by injection of a serially diluted antigen solutions. A serially diluted detection antibody solution was finally injected. A representative case of c-PSA was shown in Figure S12. We first injected 20 μg/mL capture antibody for 5 min. Serially diluted c-PSA and regeneration solution (0.2M Glycine-HCl, pH 2.0) were injected alternatively in same channel, the resultant SPR was fitted to give B values. The fitted values of B (S^{-1}) were linearly fitted, resulting in $k_a = 0.0128\ \mu g^{-1}\ ml\ s^{-1}$, $k_d = 0.0032\ s^{-1}$ and $K_D^{-1} = K_A = k_a/k_d = 4\ \mu g^{-1}\ mL$.

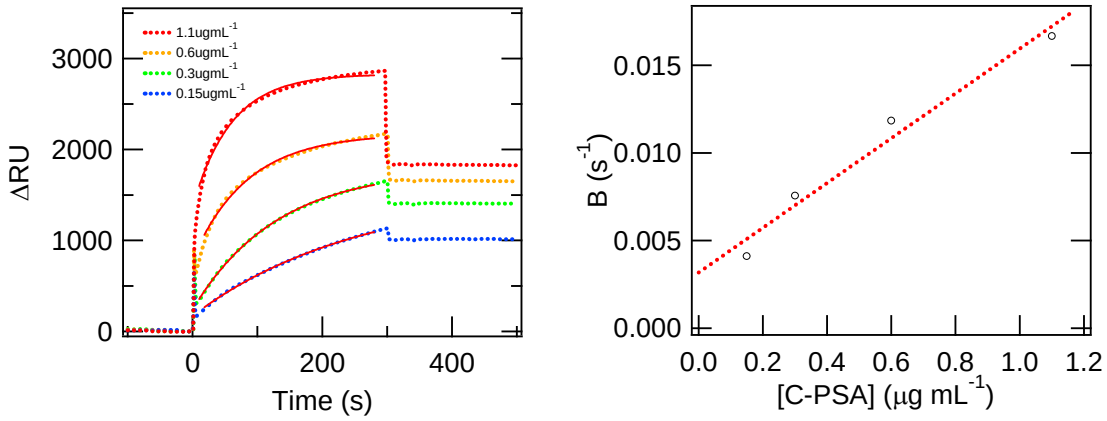


Figure S12. Representative case of dissociation constant determination of affinity pairs (capture antibody- c-PSA antigen). See more results in Table S10.

Table S10. Clinical relevance of tested tumor markers and their dissociation constants determined by SPR.

Marker	K_{D1} [a] (M)	K_{D2} [a] (M)	C_{opt} [b](μg/mL)		LOD		Corresponding cancer type
			iPDMS	NC	iPDMS	NC	
AFP	3.3E-08	1.8E-10	0.12	0.02	10.1 pM	56.0 pM	Liver
CEA	4.5E-08	5.1E-09	0.44	0.05	5.0 pM	25.1 pM	Colon, Rectum, Stomach, Lung
CK19	7.8E-08	1.5E-08	1.6	0.7	1.7 pM	11.7 pM	Lung
c-PSA	7.4E-09	1.4E-10	2	0.35	1.1 pM	24.3 pM	Prostate
NSE	7.7E-09	6.7E-09	0.4	0.26	2.4 pM	50.9 pM	Lung, Kidney
SCC	8.7E-08	1.1E-08	0.48	0.06	0.4 pM	3.7 pM	Cervix, Vagina, Lung Squamous cell carcinoma
CA15-3	N/A	N/A	2.4	0.2	N/A	N/A	Breast, Lung, Ovary
CA125	N/A	5.9E-09	2	0.4	N/A	N/A	Ovary, Endometrium
CA19-9	N/A	1.3E-08	0.64[c]	0.07[c]	N/A	N/A	Pancreas, Colon, Rectum
CA242	N/A	2.4E-08			N/A	N/A	Pancreas, Colon
β-HCG	N/A	3.8E-09	0.16	0.04	N/A	N/A	Endometrium, Choriocarcinoma, Breast, Ovary, Testis

[a]We cannot give dissociation constants in pM to the CA antigens as well as the β-HCG because they use U/L or mIU/ml as units. [b]the Optimized concentration of detection antibodies on NC and iPDMS, respectively. [c]CA242 and CA199 shared the same detection antibody.

PM, especially on iPDMS, could save material as well as time.

iPDMS is much easier to use than NC. iPDMS has two practical advantages: (1) Due to the elastmeric nature of iPDMS, each well is tightly sealed upon clamping to sustain all the incubating and stringent washing operations. Compared with other

well-formation methods such as the cover grid method 30, this neat feature is highly desired; (2) iPDMS requires no blocking step and the washing frequency is reduced from 3 times to 1 time. These advantages stem from the intrinsic nonfouling property of poly(OEGMA) coatings (i.e., preventing NPA even under complex condition such as serum or other biological fluids). NC has three practical disadvantages: (1) Glue must be applied to seal the edge of each well because NC does not seal by itself; (2) BSA blocking procedure must be applied to both top and bottom of the 48-well; (3) More washing steps must be applied because NC requires longer washing time for buffer exchange and removal of trapped molecules due to its porous nature. Both (2) and (3) are intended to reduce NPA, which is the main contributor for noise. We have demonstrated before that BSA blocking is only of limited success.

There are other advantages that make iPDMS an ideal solid supporting material for protein microarray. First, iPDMS exhibited CV at < 10% for most tested tumor markers (see full list in Table S2), which outperformed the clinical application requirements (CV < 15%). Second, the use of iPDMS also reduced the over all cost of multiplexed ELISA. From Table 1, it was clear that iPDMS typically consumed much less capture antibodies, saving up to 8-folds, suggesting that the same amount of capture antibody could be used to produce more microarrays and conduct more assays. Therefore, the problem of batch variations due to capture antibodies will be minimized. Although iPDMS consumed more second Ab-HRP, the concentration unit is μg/mL for second Ab-HRP, not the mg/mL for capture antibodies. Thus, iPDMS reduced the overall consumption of antibodies (see Figure S13, Table S11 and S12 for detailed comparisons).

Table S11. Multiplexed ELISAs on both iPDMS and NC under the same condition.

	AFP	CEA	CK19	SCC	CA125	CA19-9
$C_{Capture\ Ab}$ (mg/mL)	0.1	0.1	0.1	0.1	0.4	0.1
C_{opt} of capture Ab for iPDMS (mg/mL)	0.08	0.08	0.1	0.1	0.1	0.04
C_{opt} of capture Ab for NC (mg/mL)	0.5	0.6	0.8	0.5	0.6	0.2
$C_{antigen}$	124.1 ng/mL	49.5 ng/mL	27 ng/mL	21.6 ng/mL	247 U/mL	174.1 U/mL
$C_{Detection\ Ab}$ (μg/mL)	0.11	0.04	0.16	0.5	0.12	0.4
$R_{iPDMS/NC}$*	7.5	5.0	4.7	3.0	0.8	0.8

* The ratio between the intensity of multiplexed ELISAs on iPDMS and NC.

In this section, multiplexed ELISAs were performed on both iPDMS and NC under the same conditions (Table S11) except that NC required an extra blocking step and more washing times. In previous experiments, iPDMS and NC were performed on their own optimized conditions and typically NC required much higher concentration of array solutions (Table S11). From Figure S13, iPDMS outperformed NC for four tumor markers, namely, CK19, SCC, AFP, and CEA. We noticed that these four markers required a much higher concentration of array solution on NC. For CA 125 and CA19-9, we used conditions that were more close to the optimal concentration for NC, however, iPDMS gave a comparable value with NC. According to the optimal condition, iPDMS

based microarray consumed only ~1/16 and ~1/250 capture antibodies (Table S12), compared with NC based microarray and 96-well ELISA, respectively. These results clearly shown iPDMS could save capture antibodies while gave the same performance, if not better.

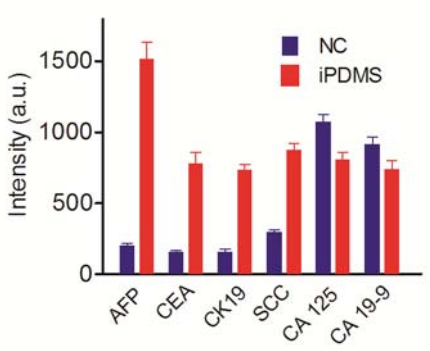


Figure S13. The comparison of intensity between on iPDMS and NC at the same condition (see Table S11).

Table S12. Comparison of consumption between traditional 96-well ELISA, NC based microarray and iPDMS based microarray.

	96-well	**NC array**	**iPDMS array**
Capture antibody (ng) *	~200	12.8	0.8
Detection antibody (ng) *	~20	0.9	1.1
throughput	96	2304	2304
Time (min)*	~30	~1	~1
automation	Yes	Difficult	Yes

* All were calculated as the average value of each well for 96-well plate, and each spot for NC and iPDMS array, resepectively.

[Details of calculation]: for 96-well plate, each well consumed 100 μL of capture antibody solution at 2 μg/mL, 100μL of detection antibody at 200 ng/mL. Each plate required on an average two days of operation time.

For PM: each well has 24 dots, each dot consumes 10 nL of printing solution on iPDMS and 20 nL on NC substrate, which had an averaged concentration at 0.64 mg/mL for NC and 0.06 mg/mL for iPDMS. Each well consumed 100μL of detection antibody solution, which had an averaged concentration at 2.15 μg/mL for NC and 2.56 μg/mL for iPDMS. Note the 2.56 μg/mL was the concentration typically used in detecting positive serum. Each array required on an average two days of operation time.

PDMS vs. p(OEGMA) coated iPDMS.

A comparison between PDMS and p(OEGMA) coated iPDMS (abbreviated as iPDMS) was made here. Six Capture antibodies were first print on both iPDMS and PDMS. Then, the PDMS was treated with a similar sandwich procedure of iPDMS except that a BSA blocking procedure was applied before target protein incubation. Figure S14 indicated that there was almost undetectable intensity of arrays on PDMS while there was over 100 folds higher intensity detected from iPDMS. We believed this was due to PDMS's low specific surface area, hydrophobicity and poor protein immobilization capacity. As we indicated in the manuscript, Nitrocellulose is the one of

the most widely used substrate in PMs research and commercial usage (i.e., Whatman FAST slides, R&D Systems Proteome Profiler Antibody Arrays, and GENTEL Symphony Array services) for its low cost and high protein capacity, which is the main reason we picked it as a control material for iPDMS study.

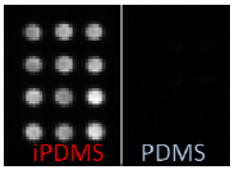


Figure S14. A local view of both iPDMS and PDMS array areas without grayscale adjustment. See text for experimental details.

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