

Fig. 1. Exemplary device and methods of collecting exhaled breath condensate (EBC) using a SiEBC (Single-drop EBC Collector/Analyzer)

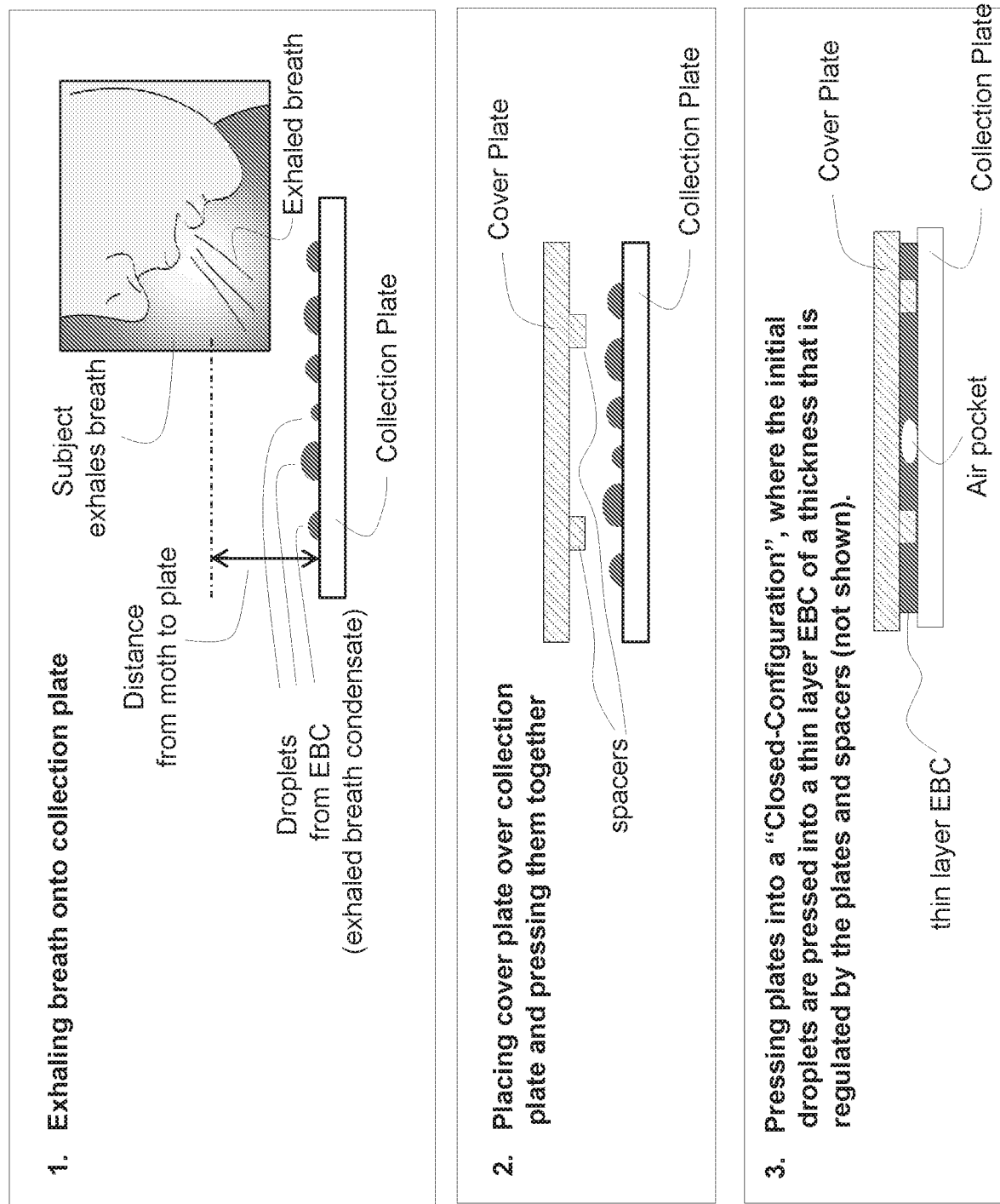


Fig. 2 Different formations of EBC at closed configuration of SiEBCA depends on spacer height

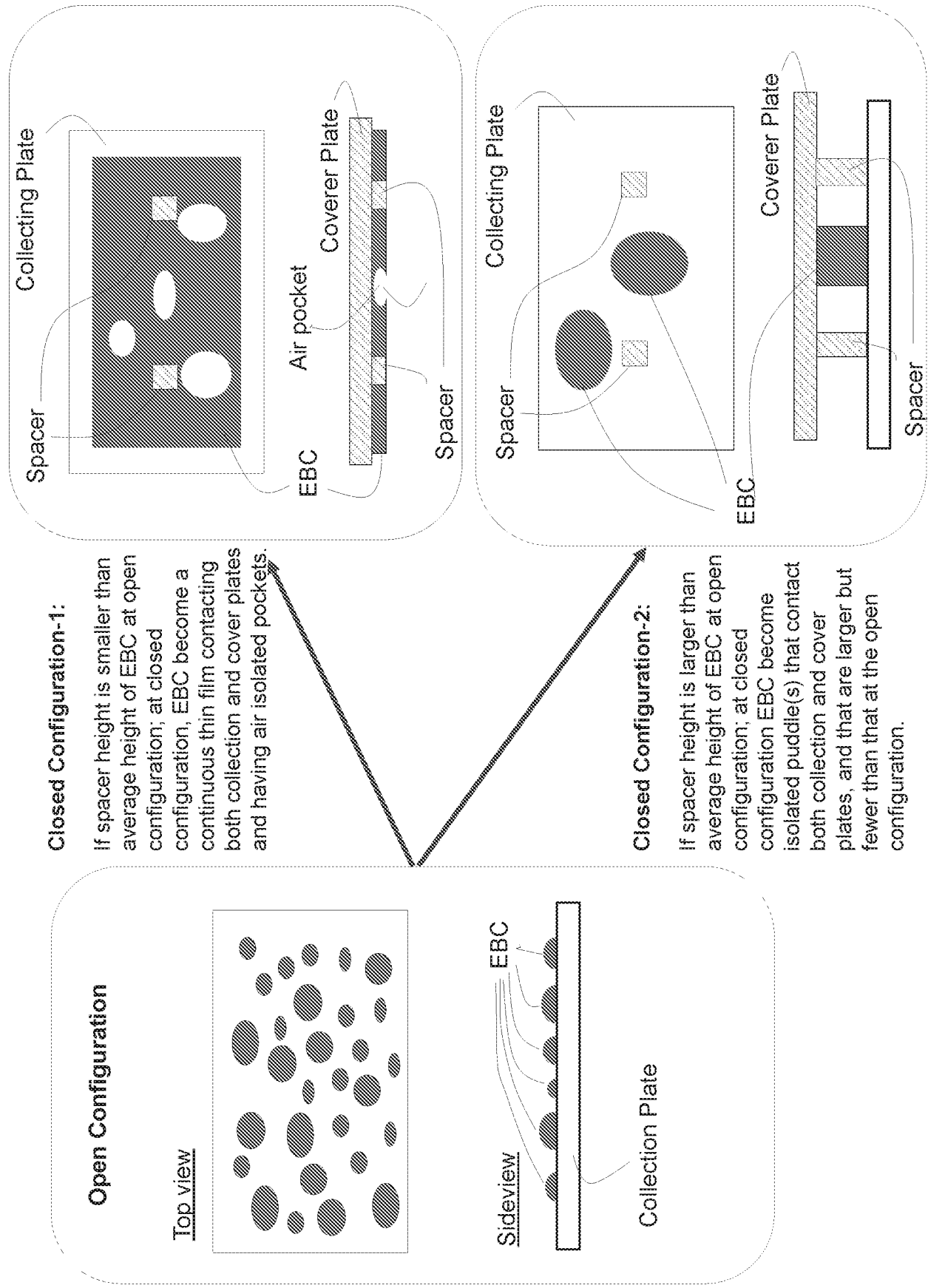


Fig. 3. An illustration of a SIEBC (Single-drop EBC Collector/Analyzer) embodiment.

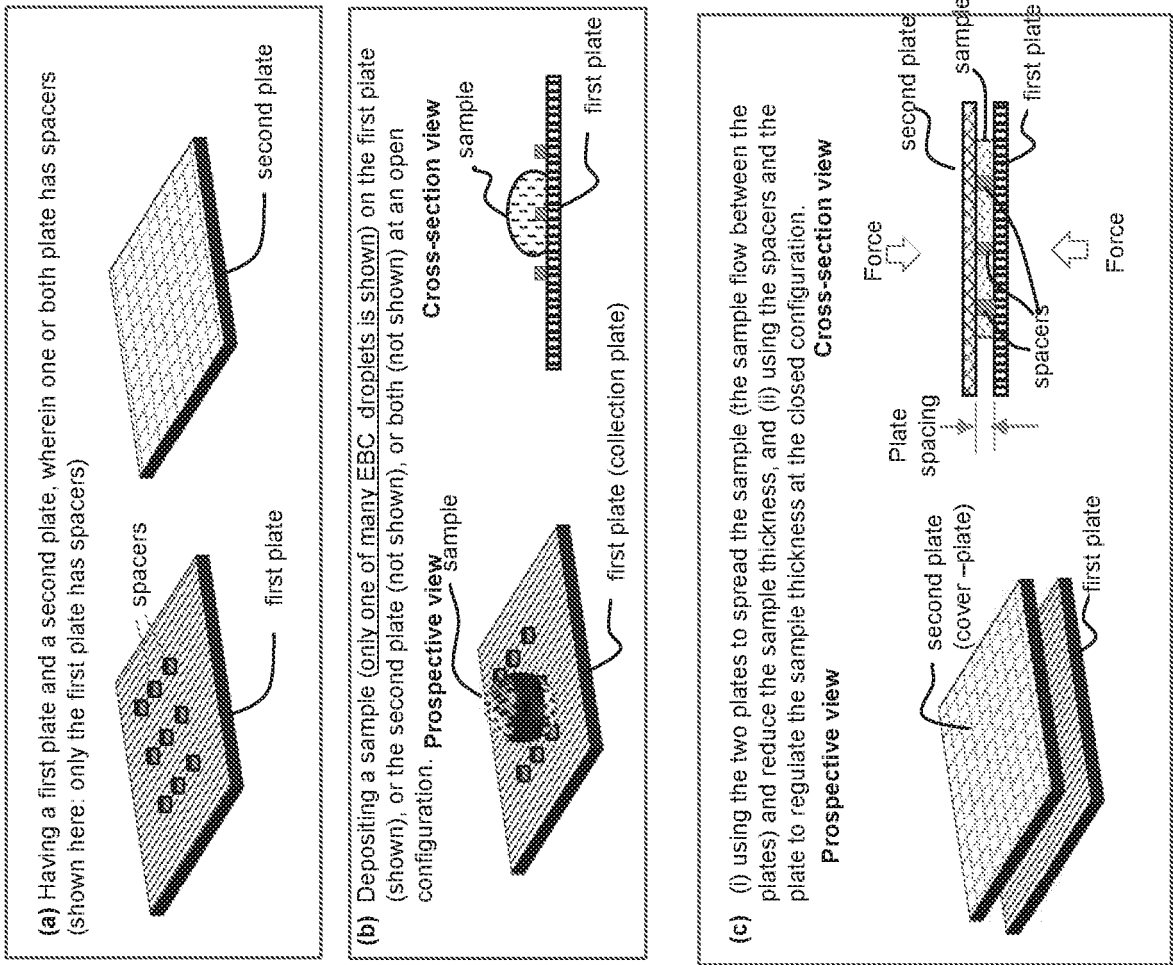


Fig. 4

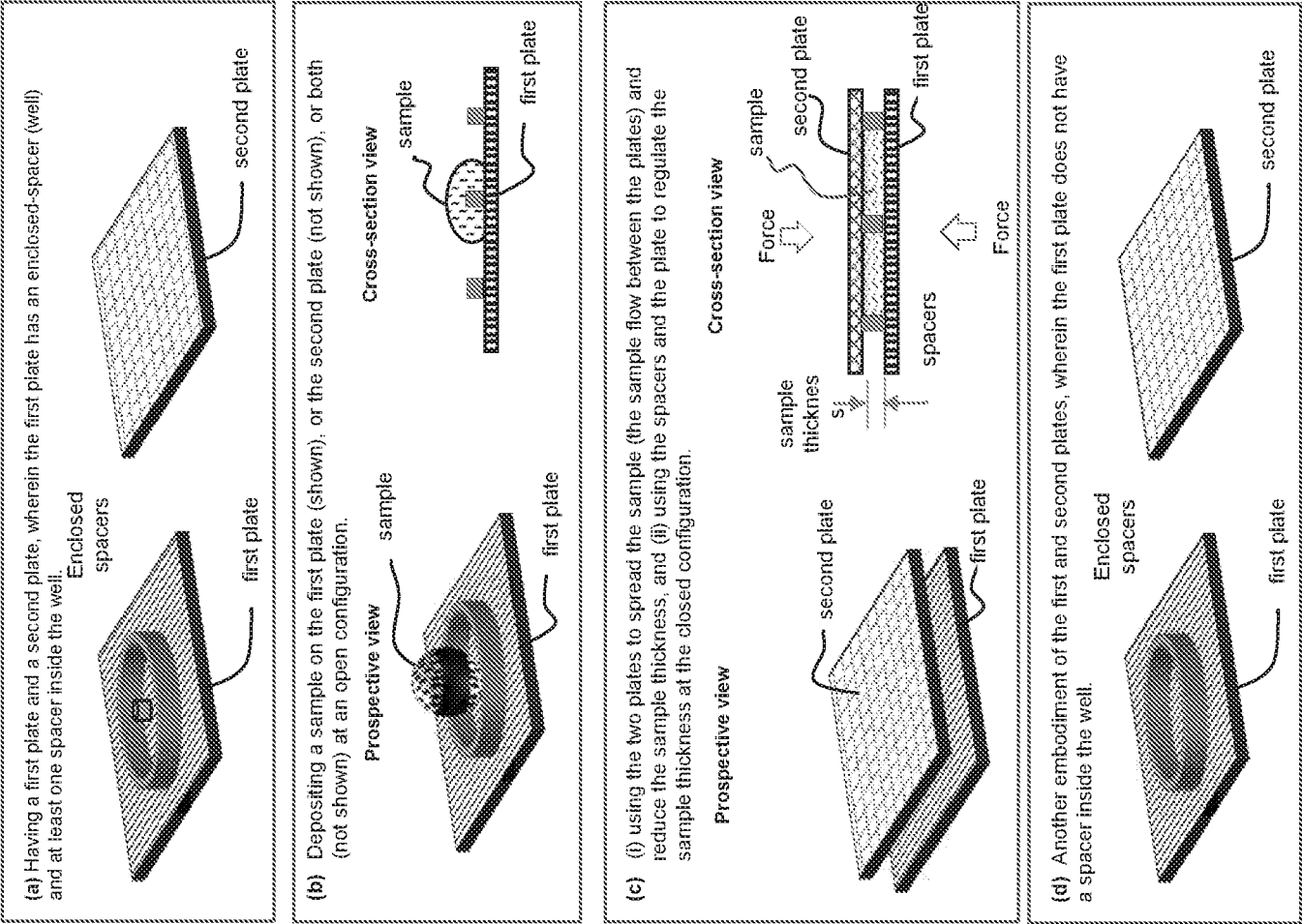


Fig. 5. Methods of pressing the plates of SiEBCA by human hand

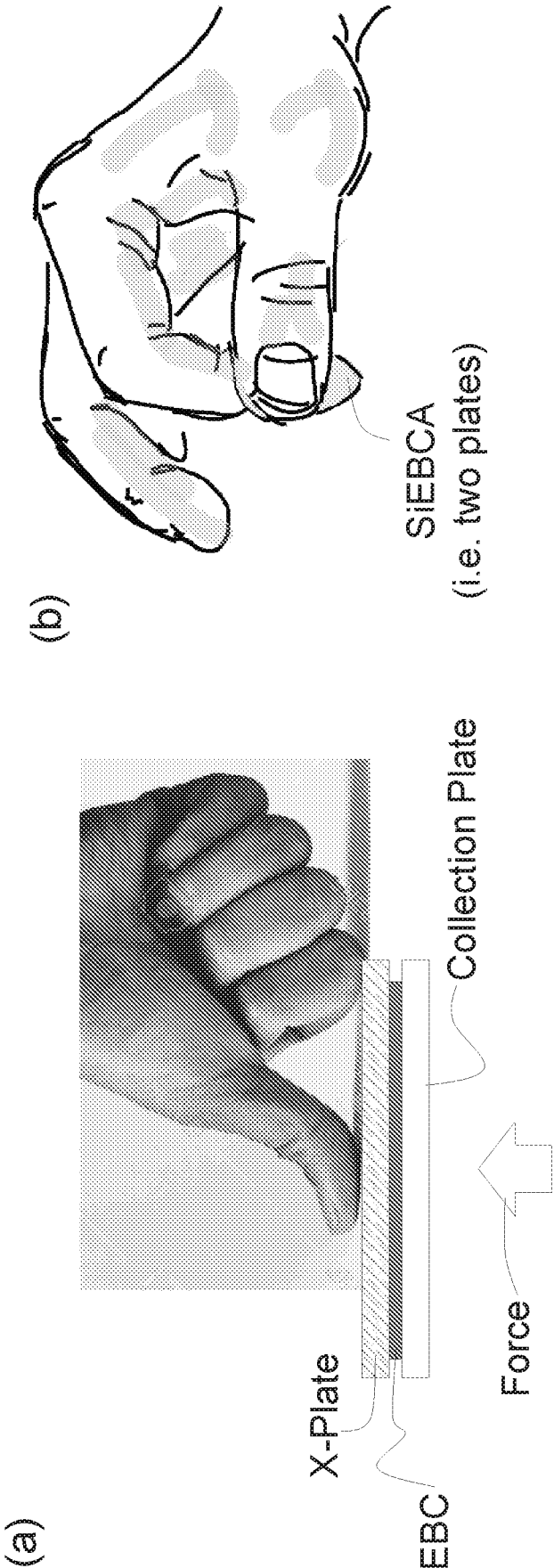
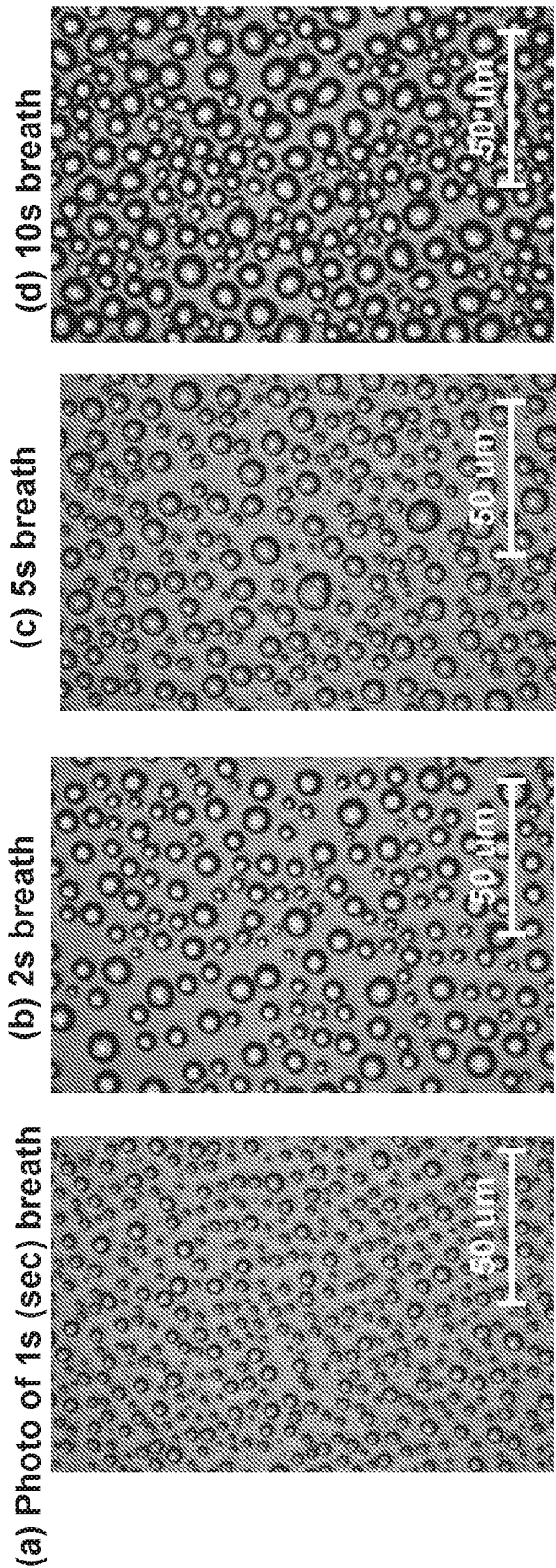


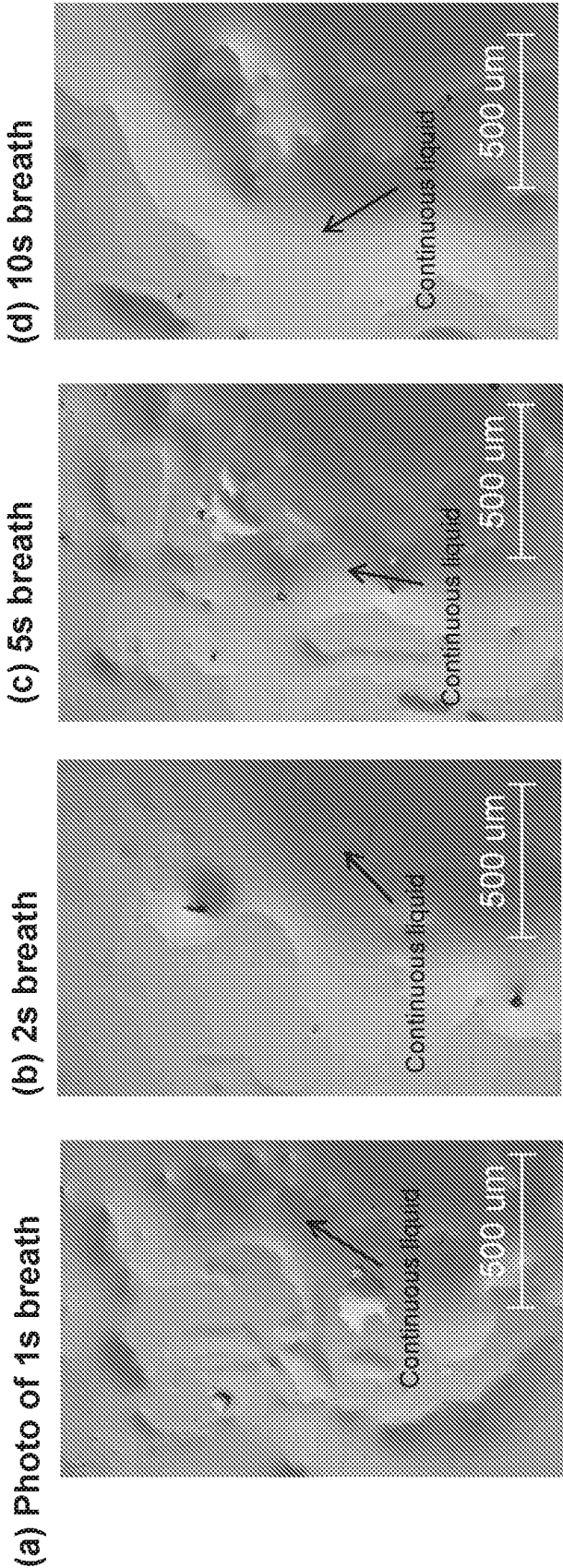
Fig. 6. Experimental data of EBC Droplets sizes and density on the collecting plate
(untreated PMMA film) at the “open configuration”



	Average droplet size (um)	Calculated Average droplet height (um)	Average droplet volume (pL)	Droplet amount density (/mm ²)	Volume density (pL/mm ²)	Total liquid surface area on 1mm ² PMMA area (mm ²)
1s breath	4.2	1.2	0.008	22000	172	0.4
2s breath	5.9	1.7	0.022	11500	250	0.42
5s breath	7.1	2	0.038	13000	491	0.7
10s breath	7.0	2	0.036	14000	507	0.7

- Breath onto *untreated PMMA collection plate.*
- Calculated Average droplet height is based on the wetting contact angle of water on PMMA.
- Volume density = Average droplet volume x Droplet amount density

Fig. 7. Experimental data of EBC droplets sizes and density on collecting plate (treated PMMA film) at the open configuration of the plates

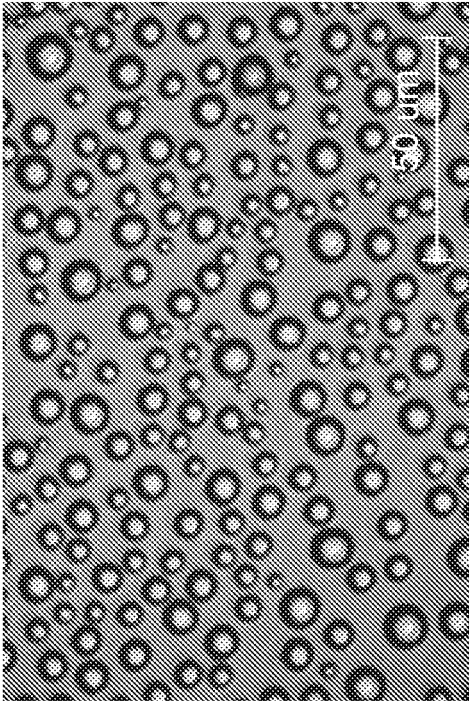


	Form Mostly Continuous (with holes) breath liquid film	Volume density (pL/mm ²) (Assume same as before)	Total liquid surface area on 1mm ² PMMA area (mm ²)	Calculated average liquid thickness (μm)
1s breath				
2s breath				
5s breath				
10s breath				
		172	~ 0.1	1.7
		250	~ 0.1	2.5
		491	~ 0.2	4.6
		507	~ 0.2	5.1

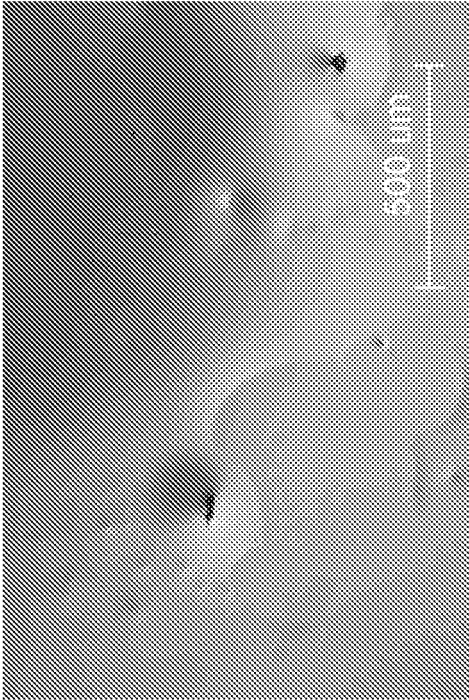
- Breath onto treated PMMA collection plate.
- Calculated average liquid thickness = Volume / Area

Fig. 8. Photography and evaporation time of EBC on untreated and treated PMMA plate at the plate open configuration

(a) 2 s breath on untreated PMMA

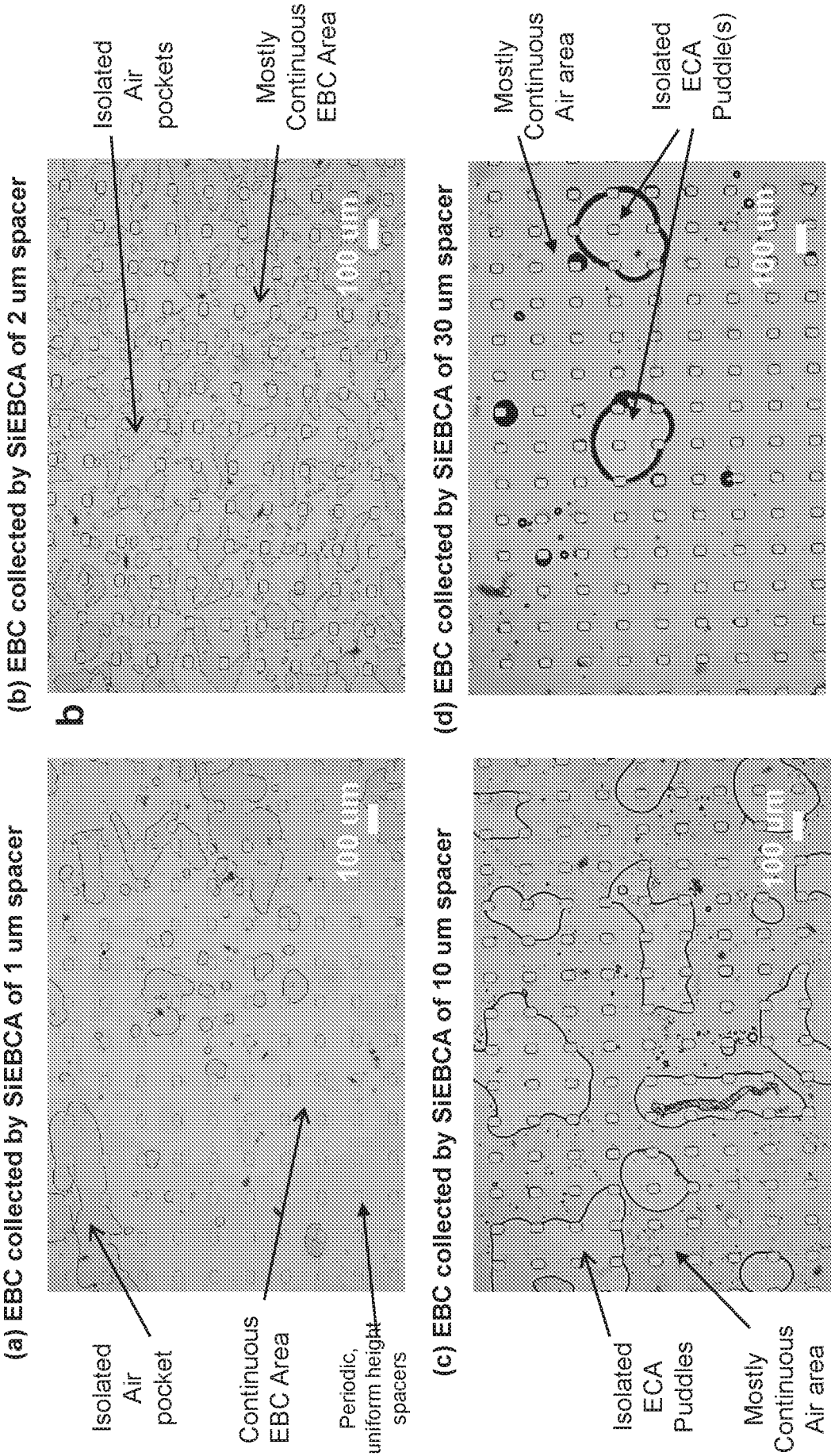


(b) 2 s breath on treated PMMA



<i>2s breath on</i>	<i>Breath Status</i>	Calculated total liquid surface area on 1mm ² collecting area (mm ²)	Experimental total evaporation time for EBC on 25mm x 25mm PMMA
Untreated PMMA	Droplets	0.42	7 s
Treated PMMA	Continuous film	~ 0.1	30 s

Fig. 9. Experimental data and Photographs of EBC collected using SiEBCA with different spacer height (1um, 2um, 10um and 30um) , respectively, with 2 s breathing time



Observations: 1. SiEBCA with 1 um gapping X-Plate have larger and continuous breath liquid area.

1. Large gapping (10 um, 30 um) X-Plate have much smaller breath liquid area.

Fig. 10. Experimental Data on EBC collected with SiEBCA of periodic spacers of 1 μ m, 2 μ m, 10 μ m and 30 μ m height, respectively.

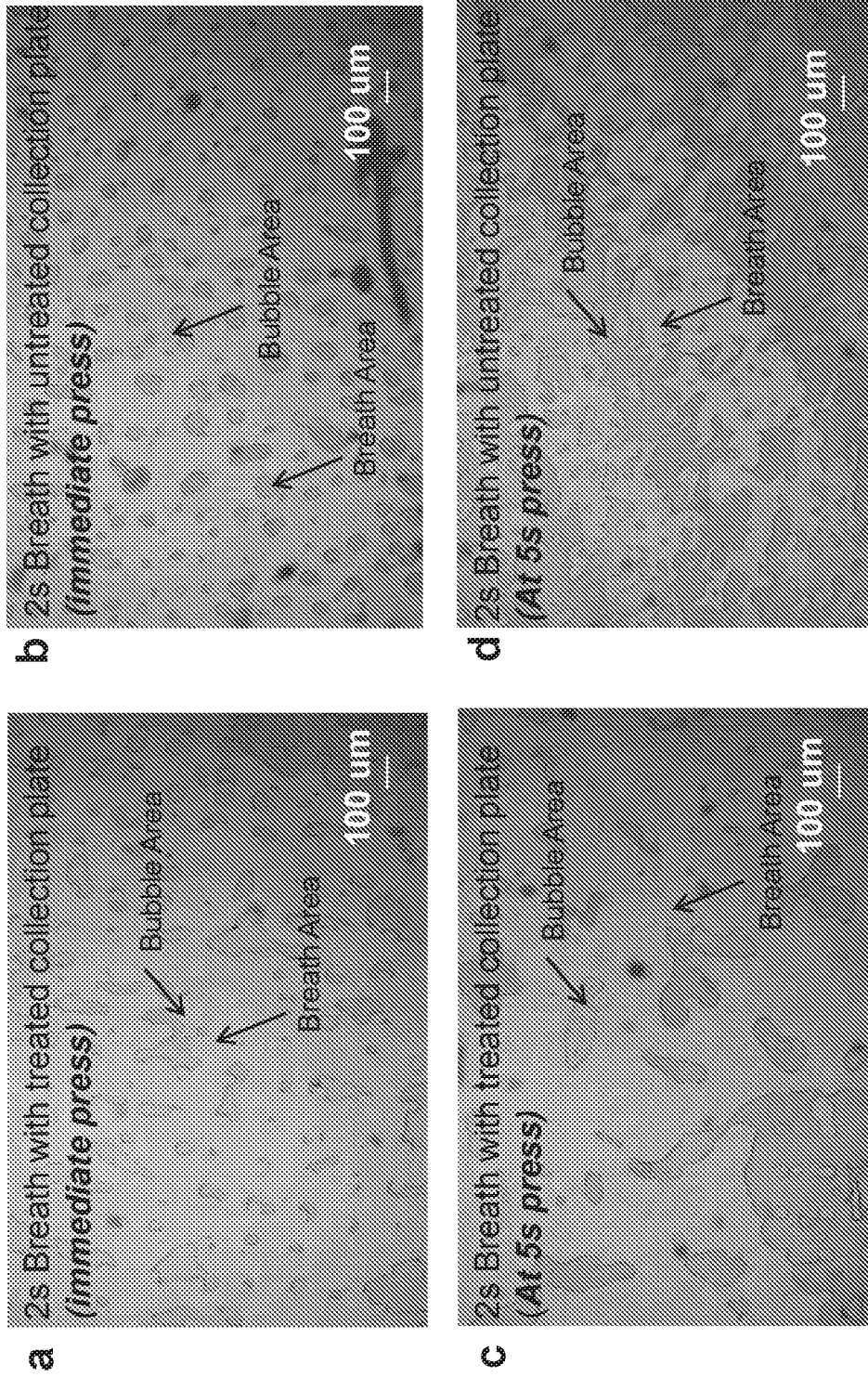
- Breath 2 s on Collection Plate (Glass 25mm x 25mm x 1mm)
- X-Plate parameter:

Material	Thickness (um)	Pillar Size (W x L) (um)	Inter Pillar Distance (um)	Ratio (IPD/ Width)
PMMA	175	30 x 38	80 / 82	2.7 / 2.2

X-Device Performance (measured in <u>2 cm by 2 cm area</u>)								
	X-Plate	Breath Liquid Status	Liquid Area (mm ²) in 25 mm x 25mm collection plate	(Liquid Area) over (Total Area - Spacer Area)	Liquid thickness (um)	Collected Breath liquid volume (uL)	Liquid thickness deviation from pillar height	Liquid thickness uniformity
1	1 um	Continuous	546	87%	1.05	0.57	5.0%	4.4%
2	2 um	Continuous	196	31%	2.38	0.47	19%	12%
3	10 um	Island	31	5%	13.7	0.42	37%	29%
4	30 um	Island	9	1%	45	0.41	50 %	35%

1. 1 um gapping X-device can collect most amount of breath liquid with good gapping uniformity.
2. X-device with gapping larger than 1um collect less breath liquid and hard to self-hold well, thus has worse gapping deviation and uniformity.

Fig. 11. Photographs of observed breath collection with treated and untreated PMMA collection plates



1. With treated (wetting) PMMA collection plates, more breath liquid can be collected both in "immediate press" and "at 5s press" cases.

Fig. 12. Experimental data on effects of (a) treated and untreated PMMA collection plates and (b) time delay in closing the cover plate on breath collection.

• *Breath 2 s on Collection Plate (PMMA 25mm x 25mm x 1mm)*

X-Plate parameter:

Material	Thickness (um)	Pillar Size (W x L) (um)	Inter Pillar Distance (um)	Pillar Height (um)	Ratio (Width / Height)	Ratio (IPD/Width)
PMMA	175	30 x 38	80 / 82	1	30 / 38	2.7 / 2.2

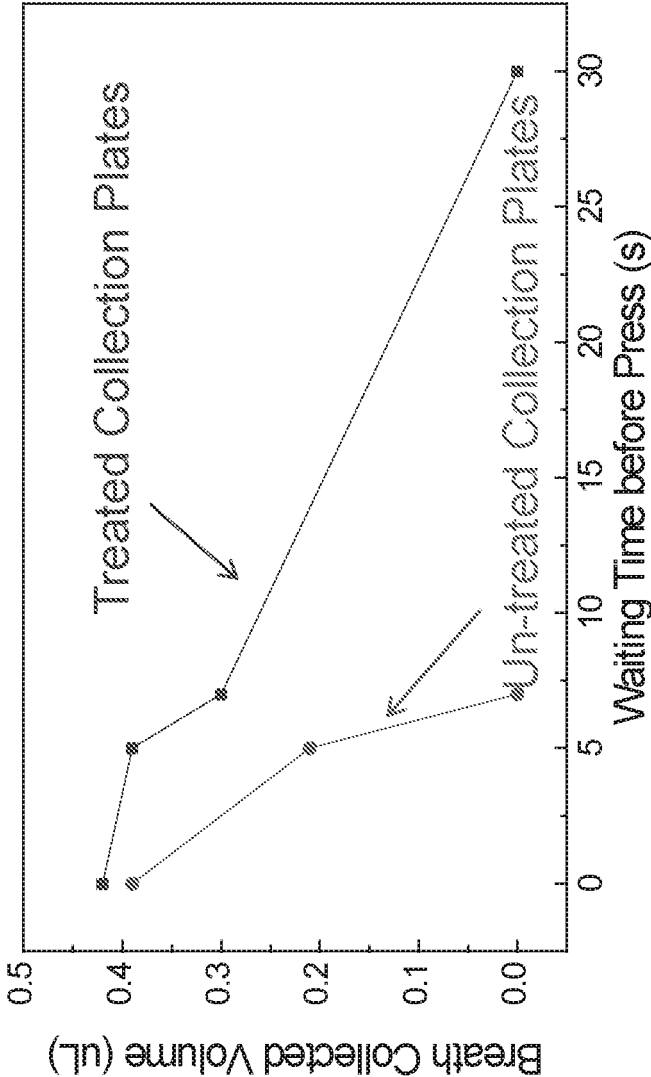
		SiEBCA-Device Performance (measured in <u>2 cm by 2 cm area</u>)						
Collection Plate	Wait time before Press	Liquid Area (mm ²) in 25 mm x 25mm collection plate	(Liquid Area) over (Total Area - Spacer Area)	Liquid thickness (um)	Collected Breath liquid volume (uL)	Liquid thickness deviation from pillar height	Liquid thickness uniformity	
Surface treatment								
1	Treated	Immediate	405	65%	1.03	0.42	3.3%	4.7%
2	Treated	At 5s	366	59%	1.07	0.39	7.4%	6.4%
3	Un-treated	Immediate	370	59%	1.05	0.39	5.2%	5.7%
4	Un-treated	At 5s	138	30%	1.52	0.21	52 %	22%

1. Compared untreated PMMA collection plate, the treated collection plates (better wetting) collect more breath liquid in both delay times of "immediate press" and "at 5s press".
2. Using the untreated collection plates (less hydrophilic), at 5s delay time for covering, almost a half of the liquid was evaporated.

Fig. 13. Collected breath volume vs. time with treated and untreated collection plates (Wait different time before pressing)

Collection - Plate parameter:

Material	Thickness (um)	Plate size (mm)
PMMA	250	25 x 25



- Breath time: 2 s
- Volume is measured by SiEBCA at time t.
- With treated collection plates (wetting), more breath liquid can be collected.
- Before press, breath liquid on it could sustain longer (30 s) on treated collection plates than on untreated collection plate (7s).

Fig. 14 Experiment Data for drying time of EBC collected by SiEBCA and at “Closed configuration”

- *Breath 2 s on Collection Plate (PMMA 25 mm x 25 mm x 250 μ m)*
- *X-Plate parameter (25 mm x 25 mm x 175 μ m):*

Material	Thickness (μ m)	Pillar Size (W x L) (μ m)	Inter Pillar Distance (μ m)	Ratio (Width / Height)	Ratio (IPD/ Width)
PMMA	175	30 x 38	80 / 82	30 / 38	2.7 / 2.2

Observed drying time of EBC inside SiEBCA

Collection plate	X-Plate	Drying Time (min)	Average Drying Speed (mm^2/min)
Surface treatment	Height (μ m)		
Treated	1	70	8.9
Treated	10	45	13.9
Un-treated	1	40	15.6
Un-treated	10	10	62.5

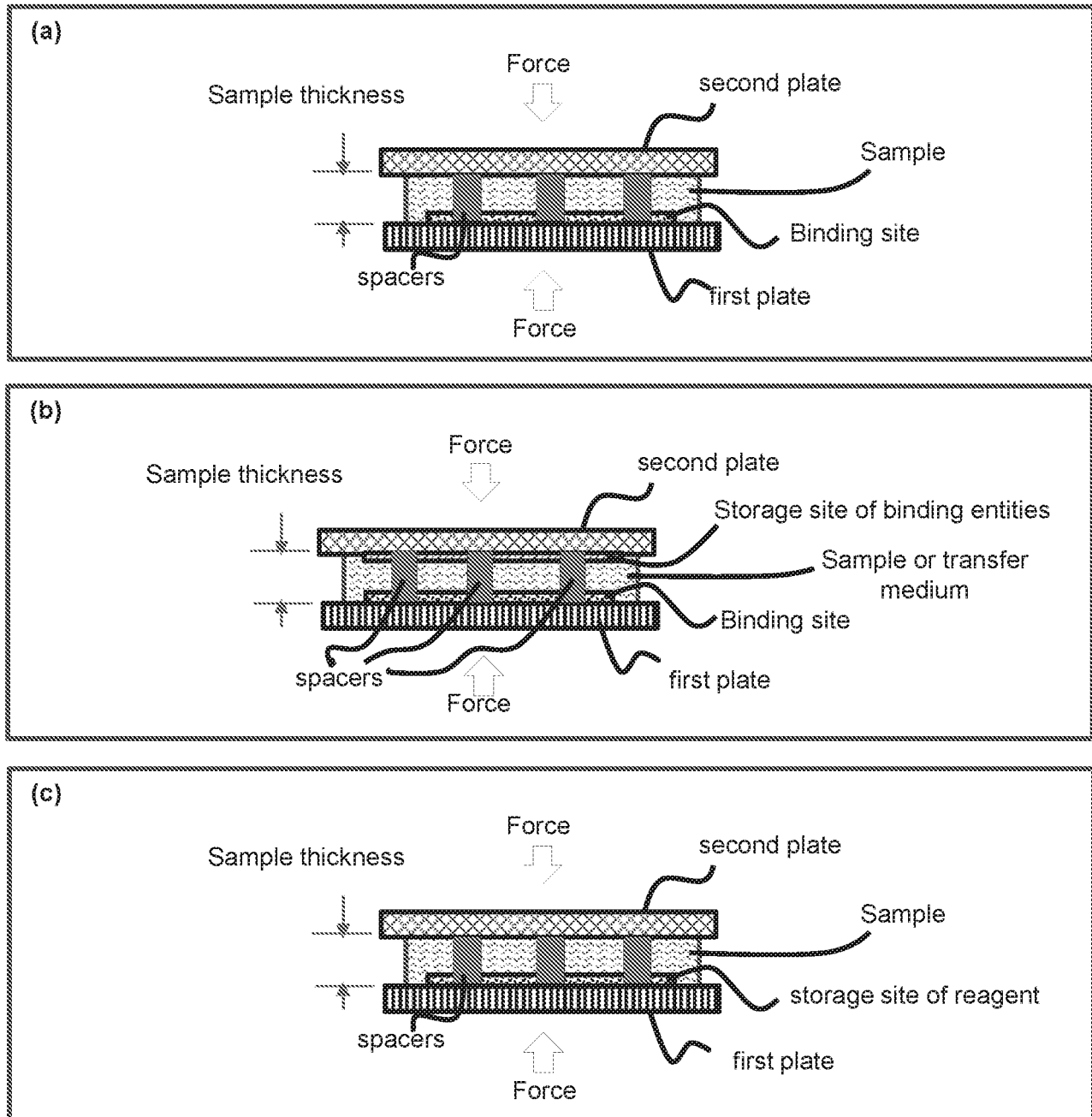


FIG. 15

Case 1. Multiplexing. One binding site for one or multiple storage sites

Different storage sites can have the same detection agent but different concentration or different detection agents of the same or different concentration.

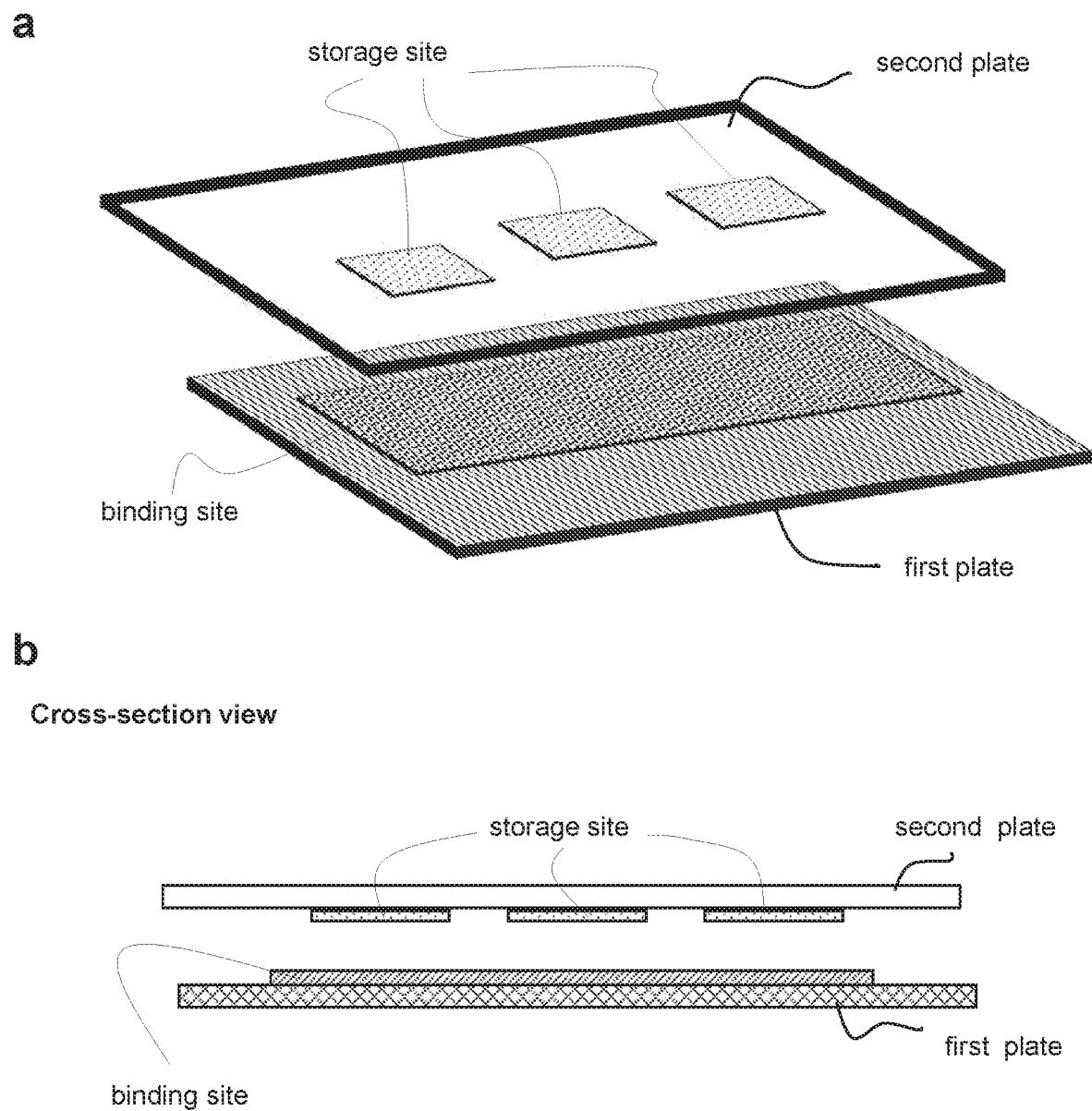


FIG. 16

Case 2. Multiplexing. One storage site for one or multiple binding sites

Different storage sites can have the same detection agent but different concentration or different detection agents of the same or different concentration.

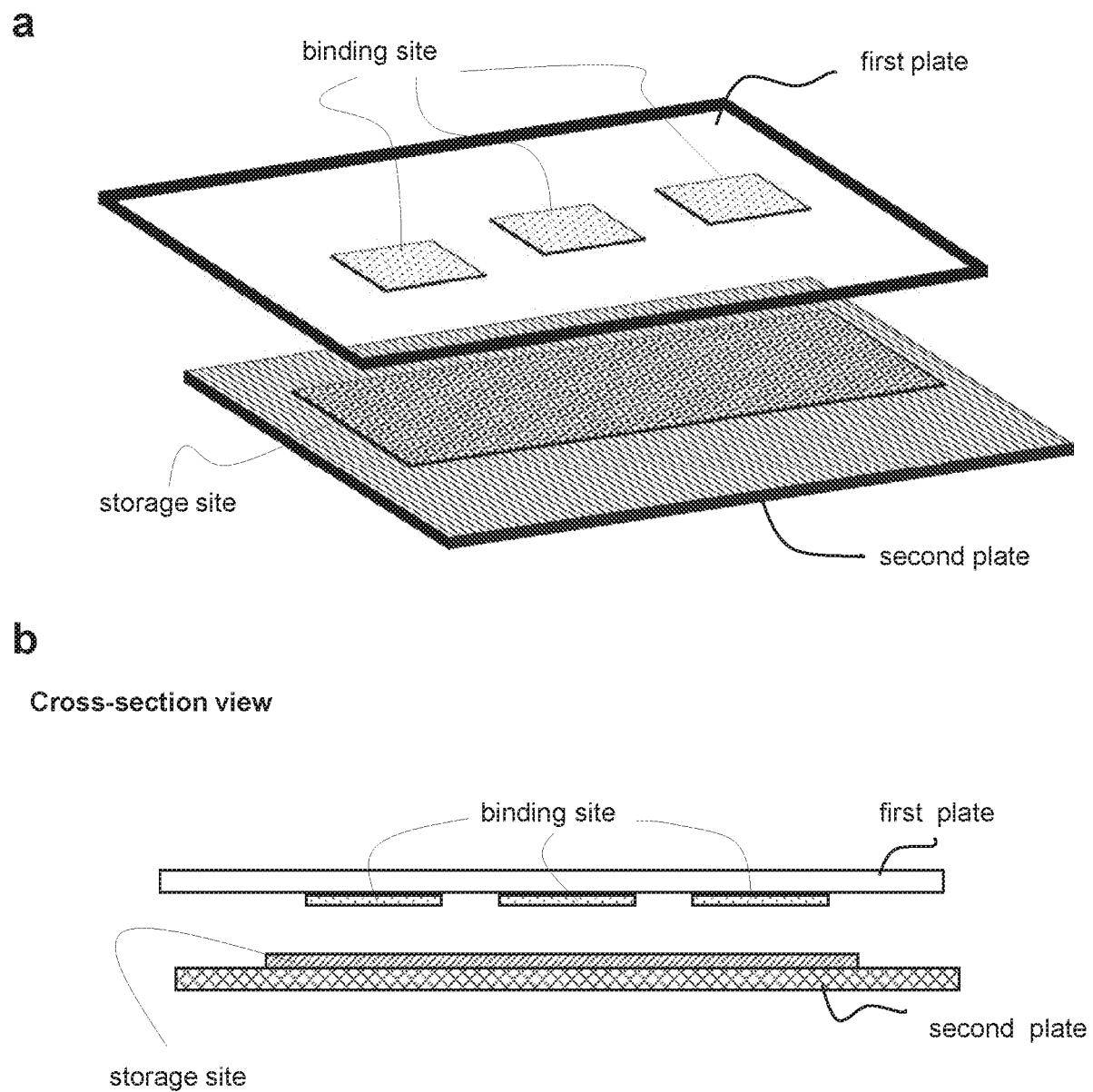
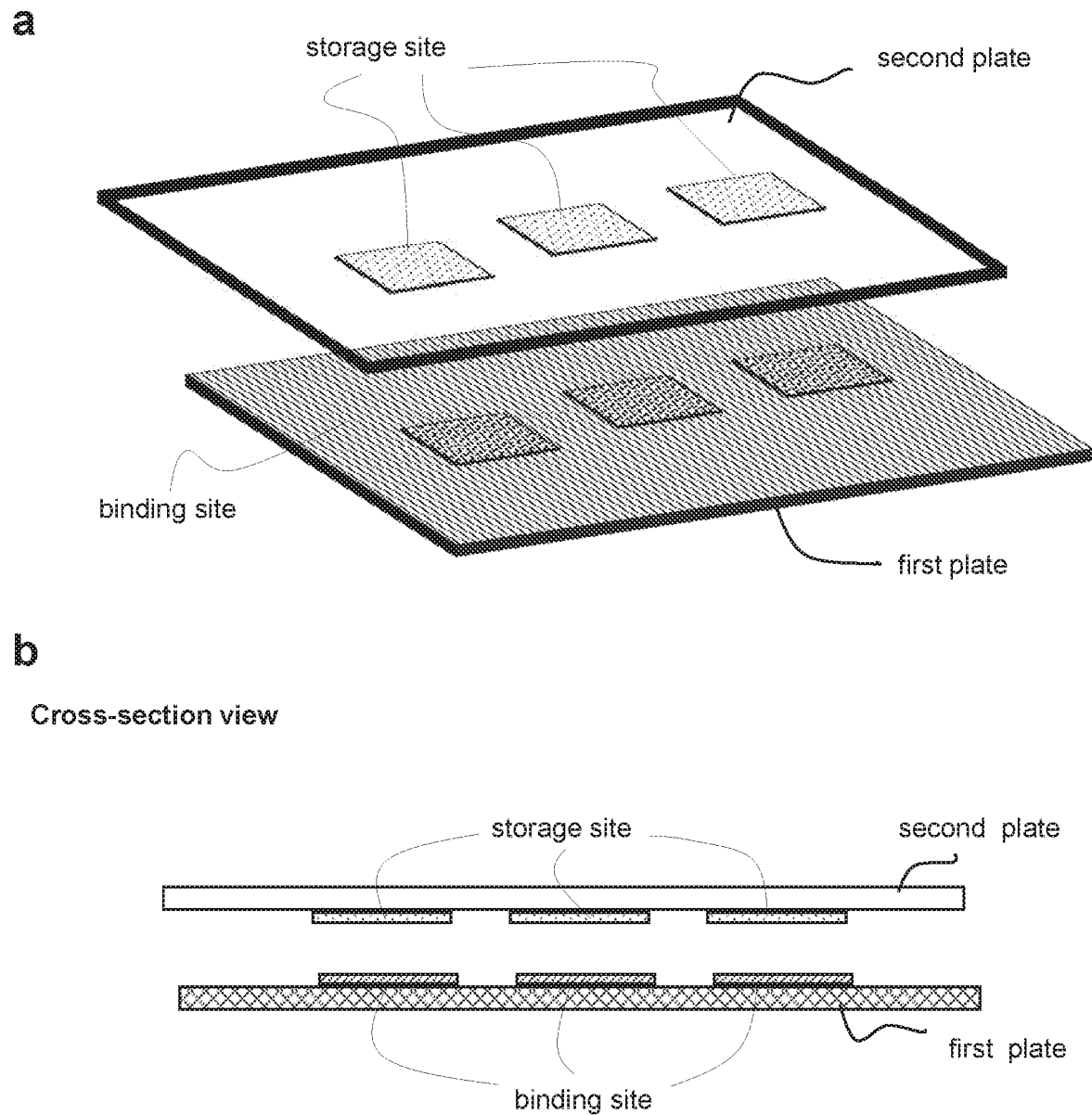


FIG. 17

Case 3. Multiplexing. Multiple binding sites and multiple corresponding storage sites

Different storage sites can have the same detection agent but different concentration or different detection agents of the same or different concentration.

**FIG. 18**