

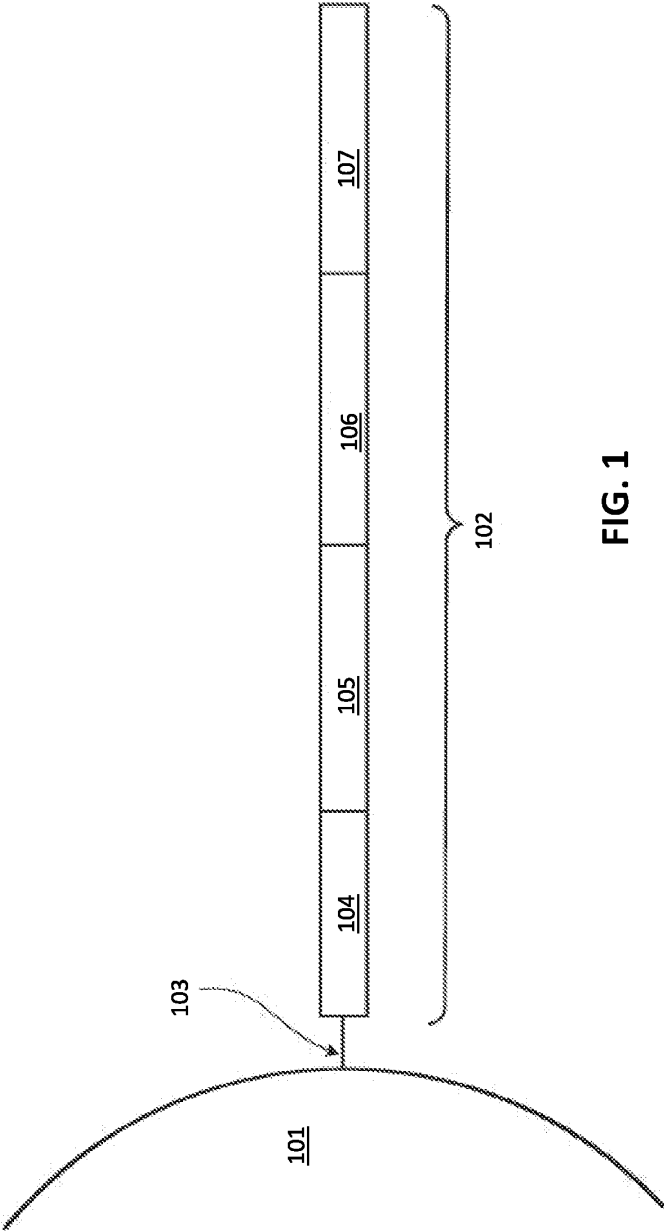


FIG. 1

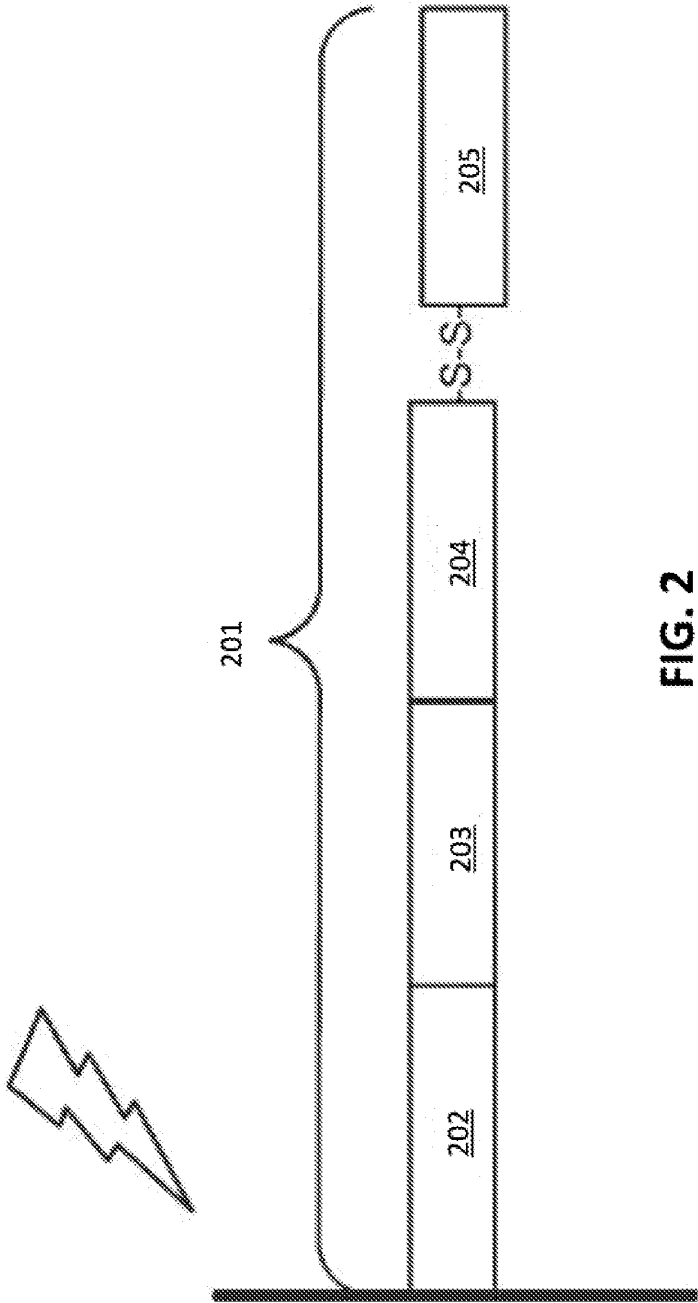


FIG. 2

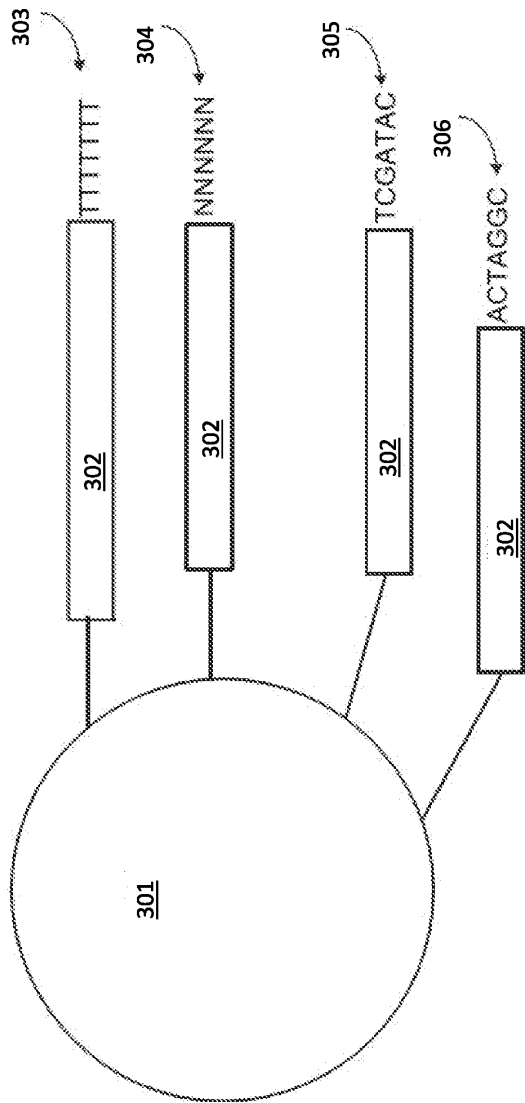
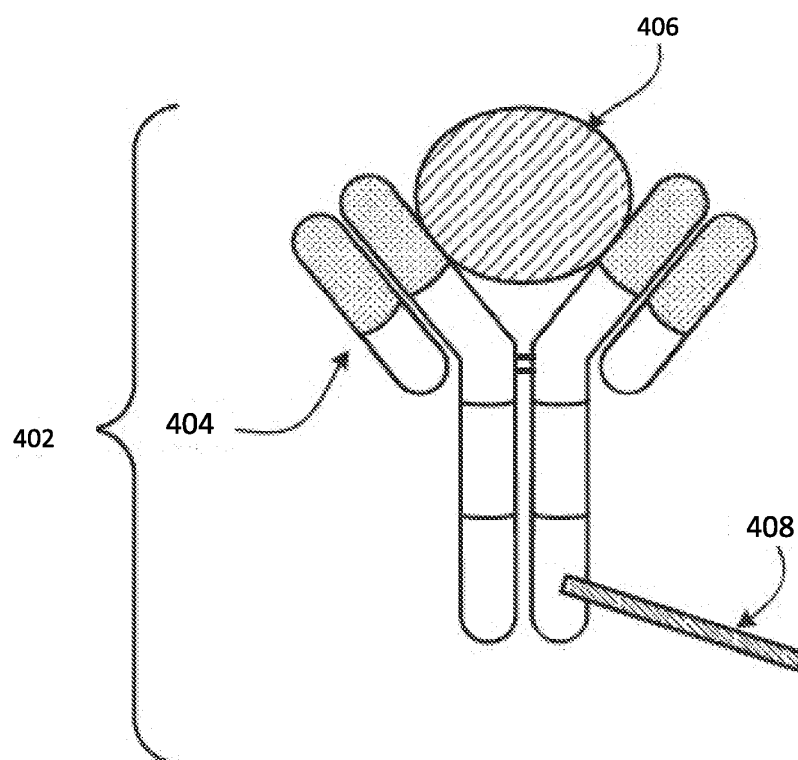


FIG. 3

**FIG. 4**

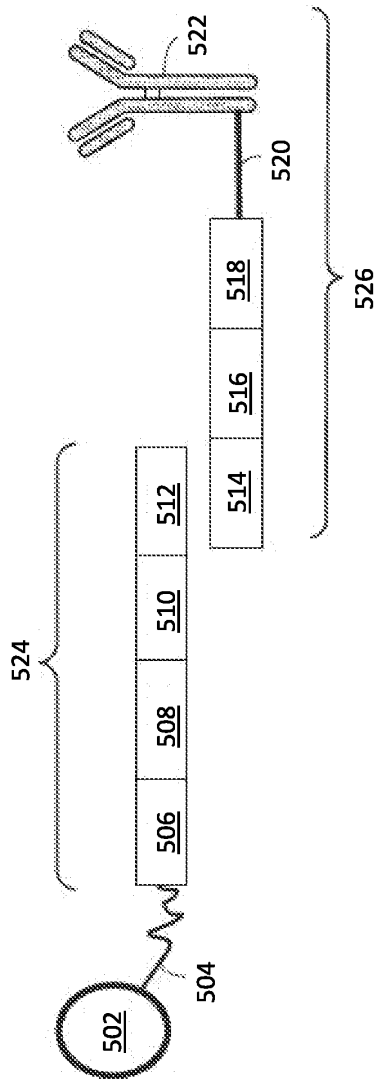


FIG. 5

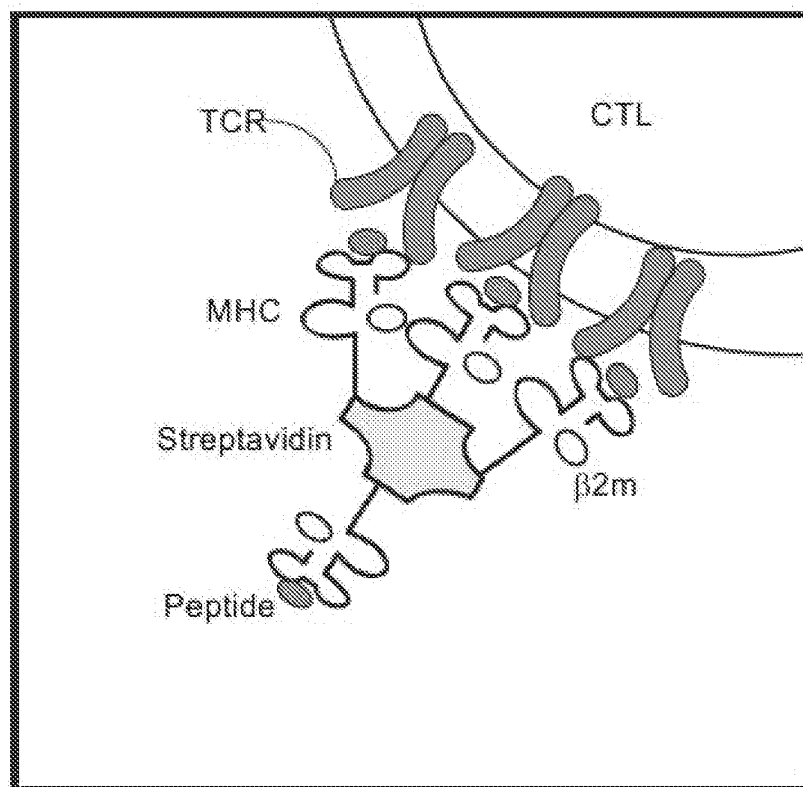


FIG. 6A

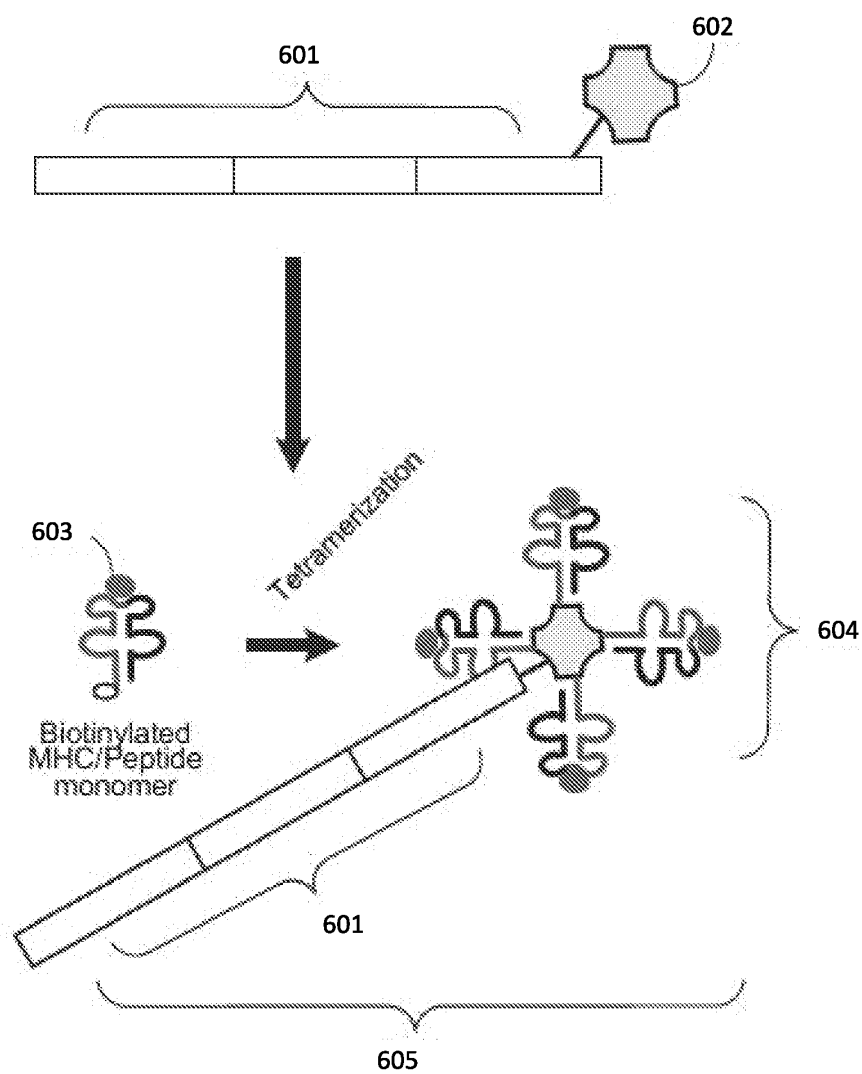


FIG. 6B

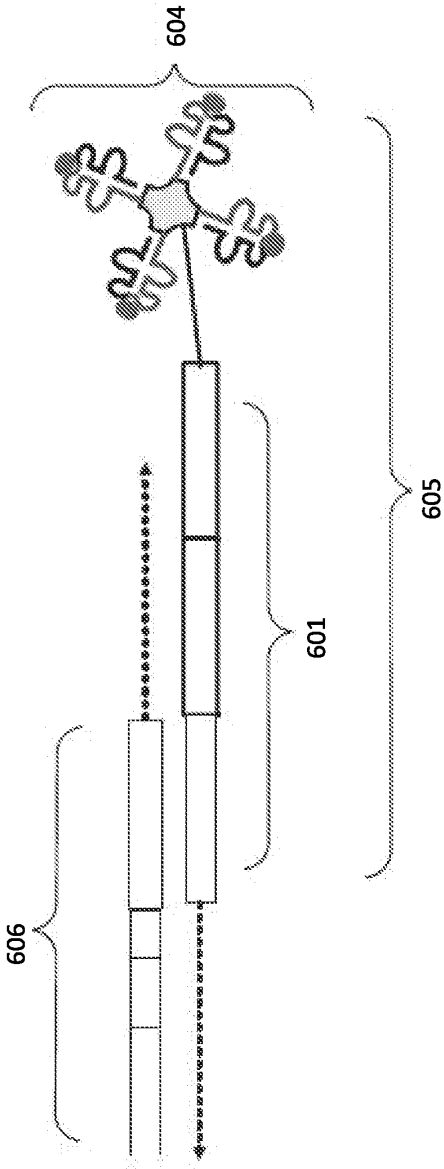
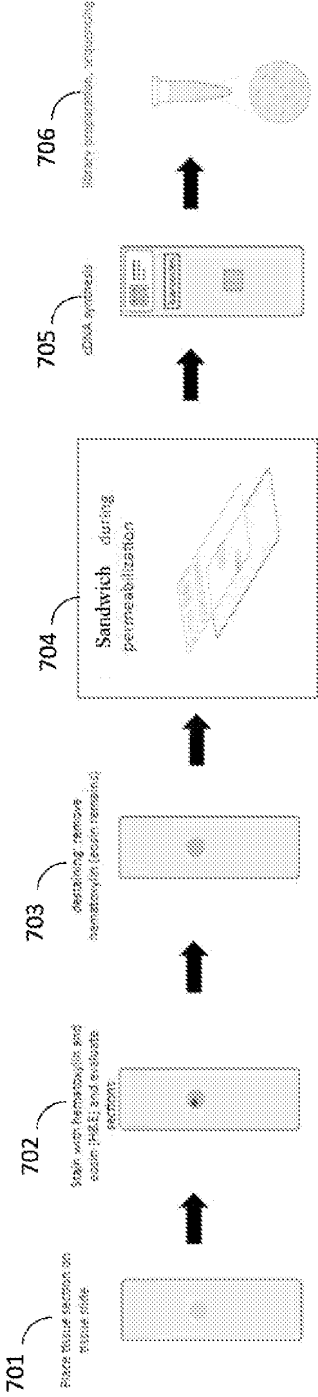


FIG. 6C



The sandwich is assembled during the permeabilization step. Permeabilization solution is introduced, the tissue is permeabilized/digested and released transcripts diffuse across the gap from the tissue to the GEX slide and bind on the barcoded oligos.

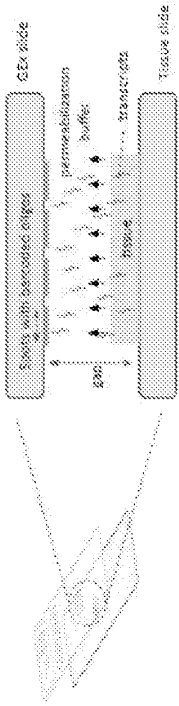


FIG. 7

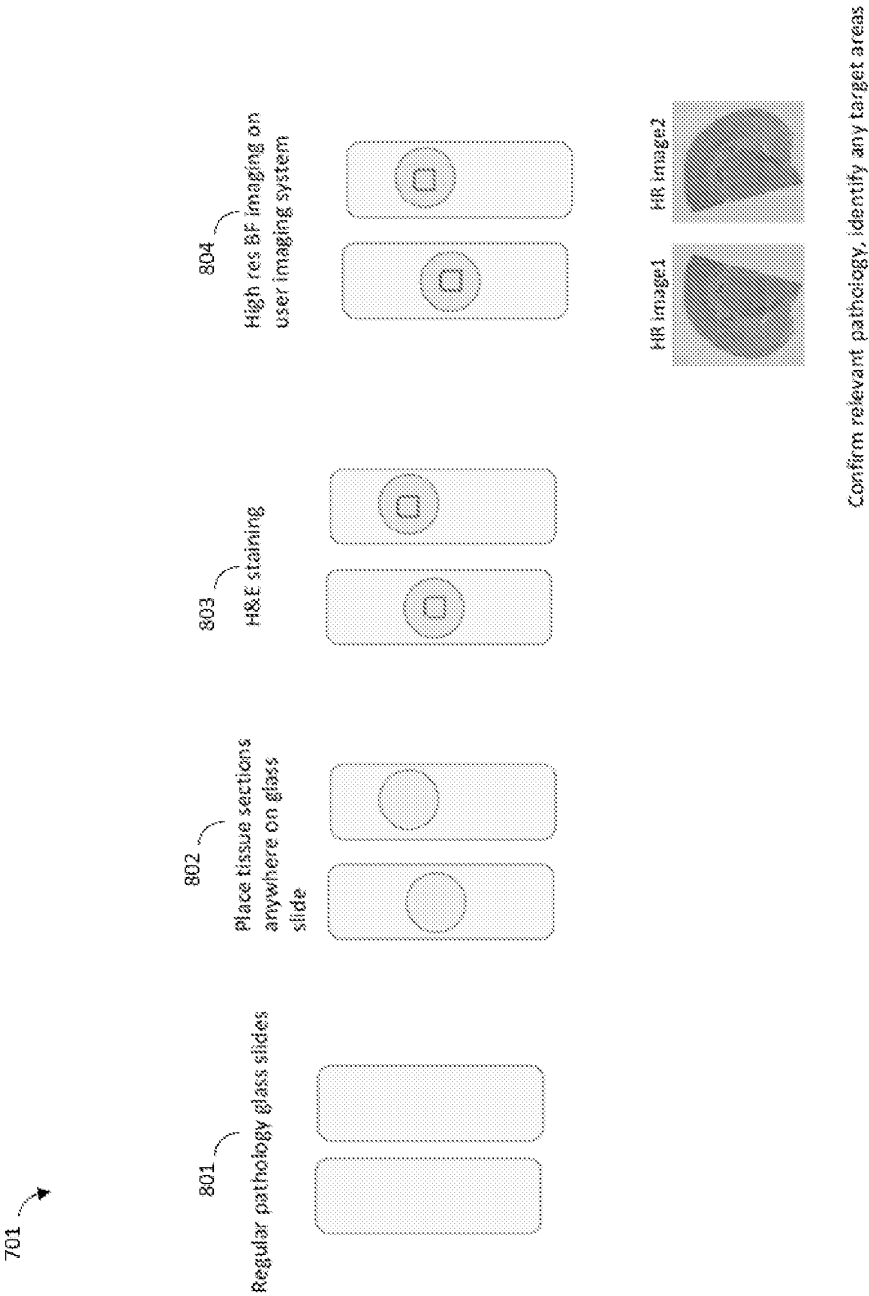


FIG. 8

704

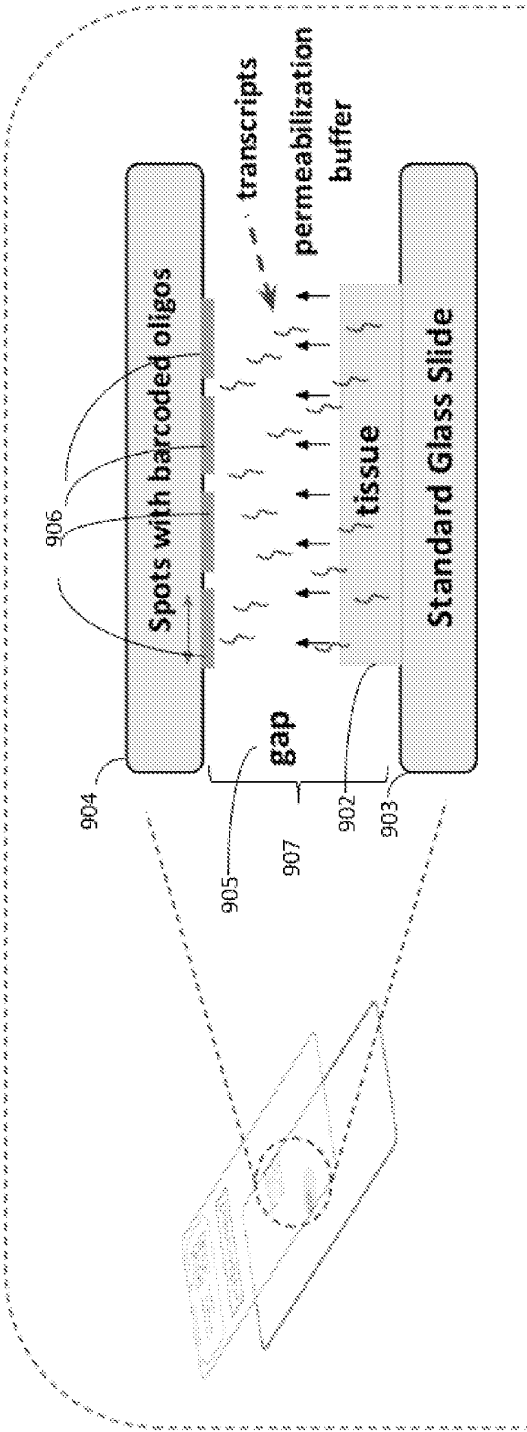


FIG. 9A

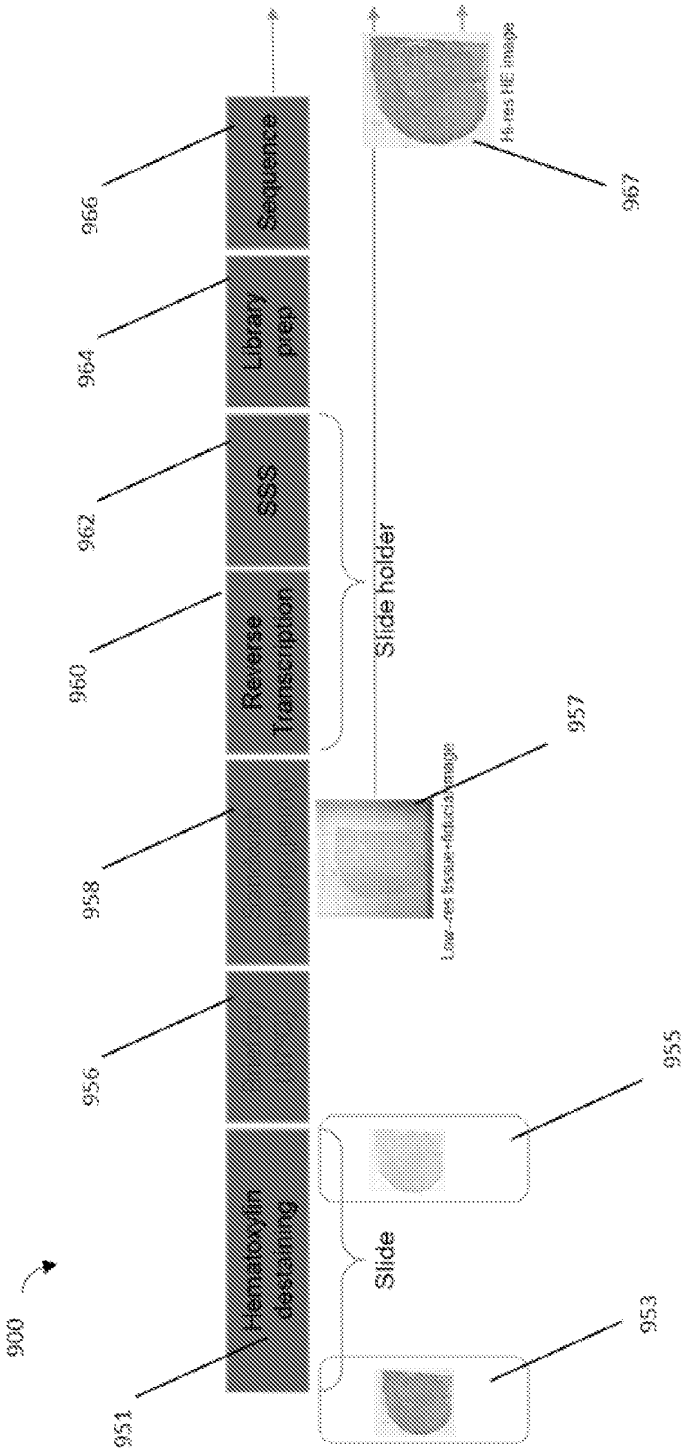


FIG. 9B

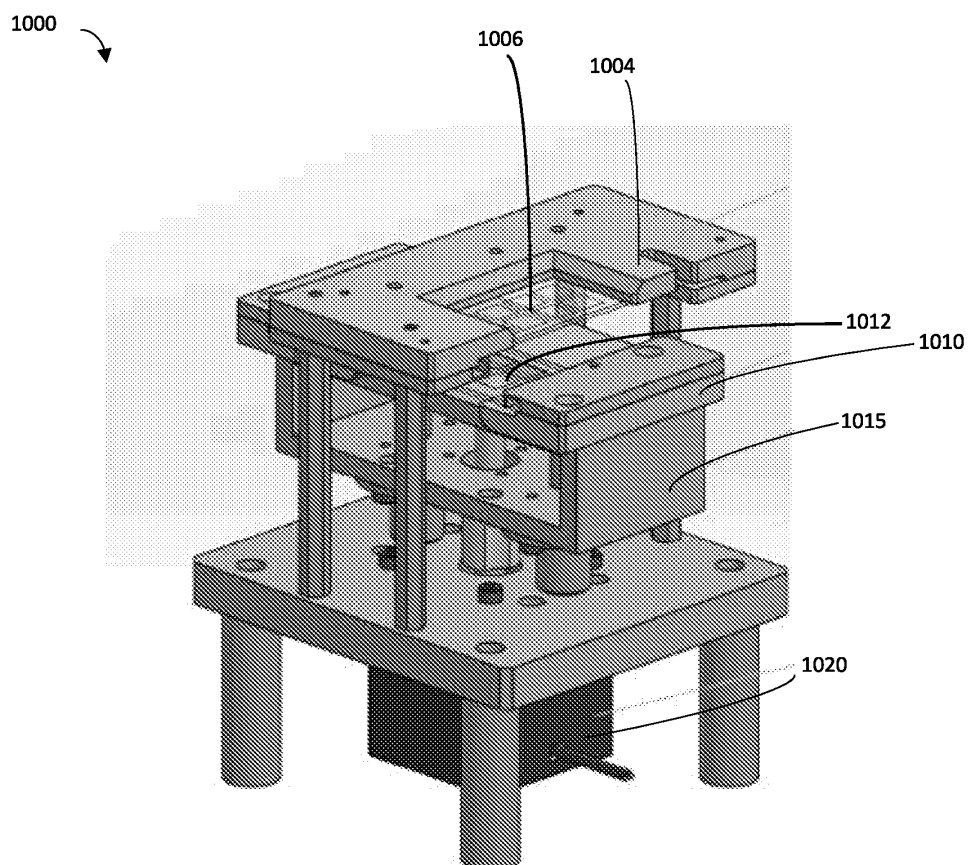


FIG. 10

1015

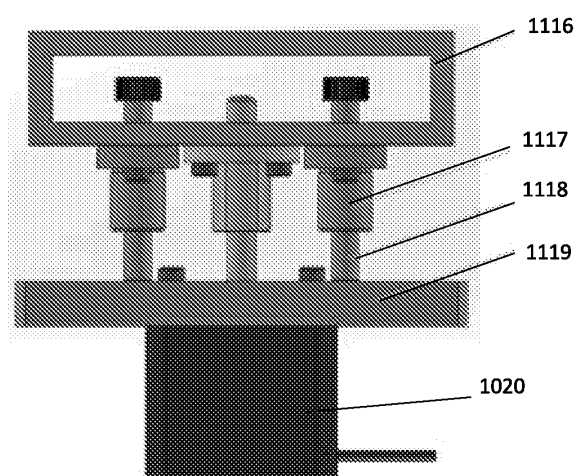


FIG. 11

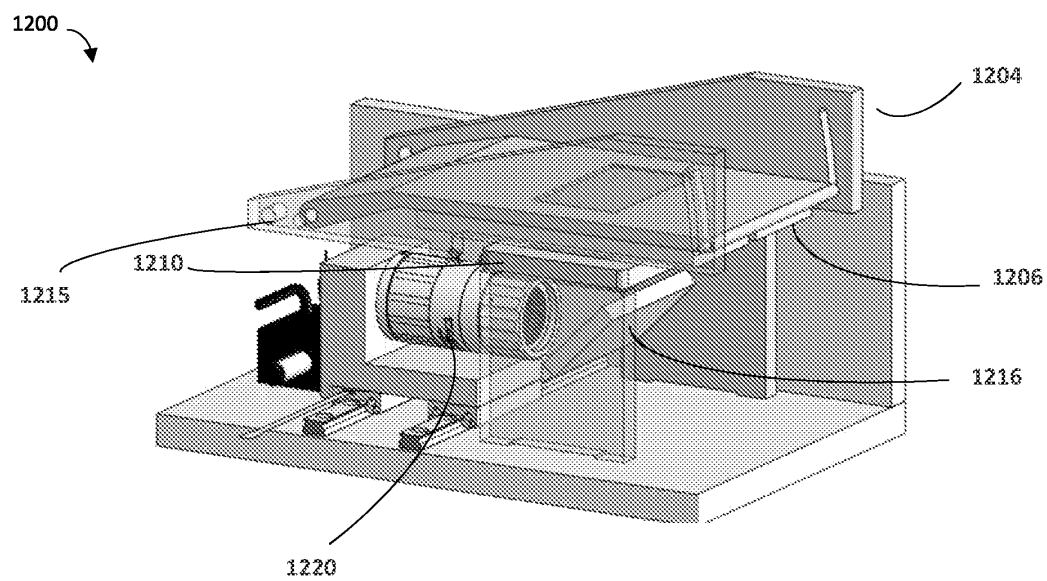


FIG. 12A

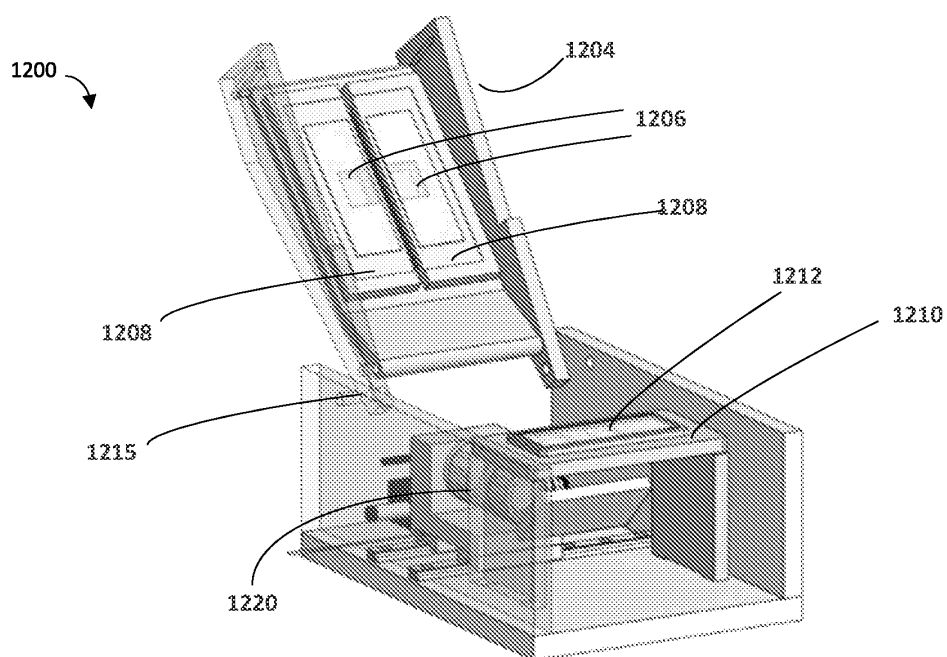


FIG. 12B

1300

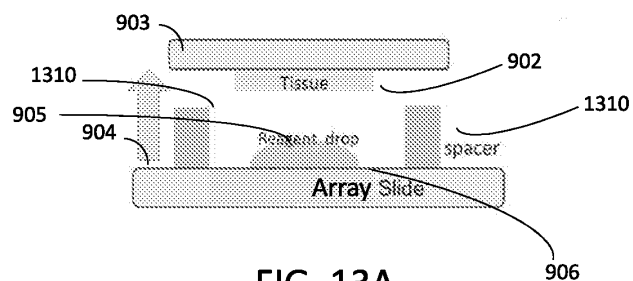


FIG. 13A

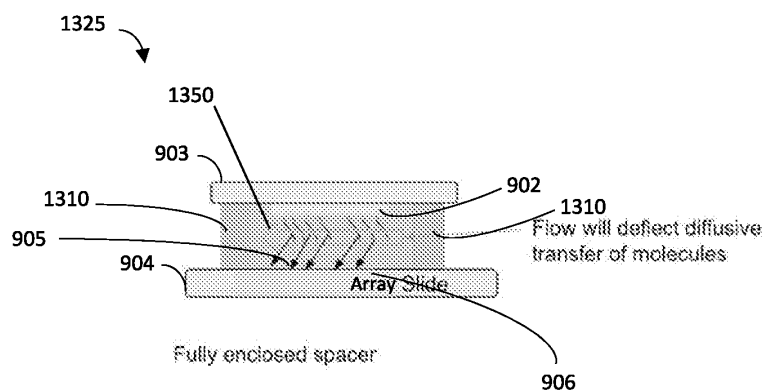


FIG. 13B

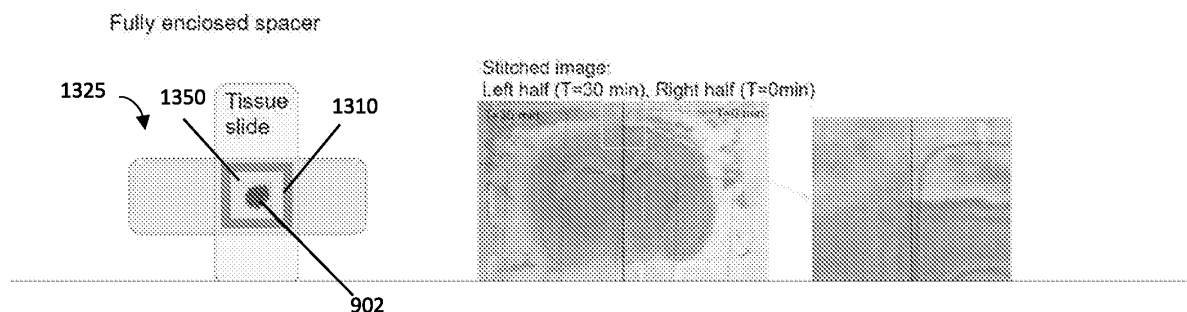


FIG. 13C

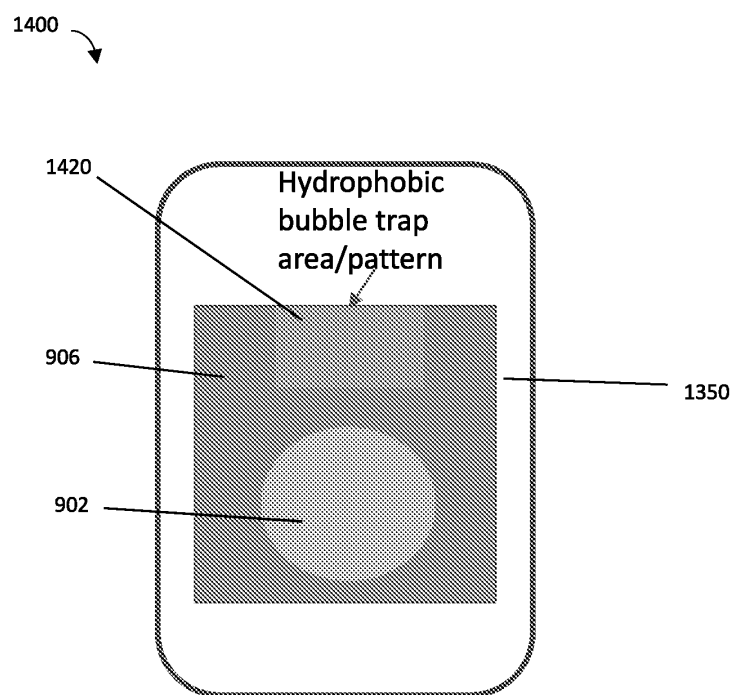


FIG. 14

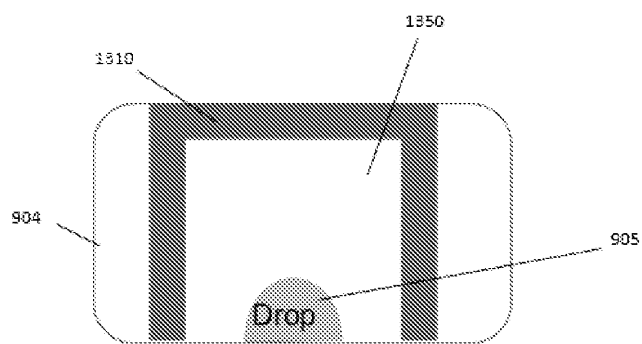


FIG. 15A

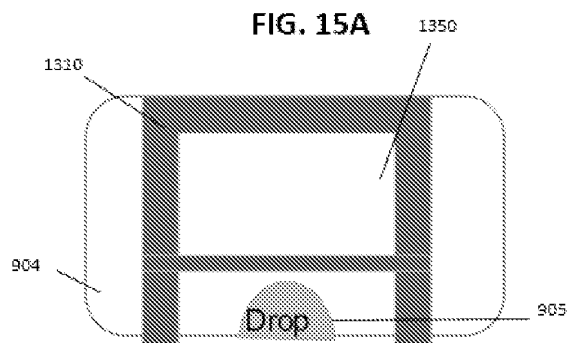


FIG. 15B

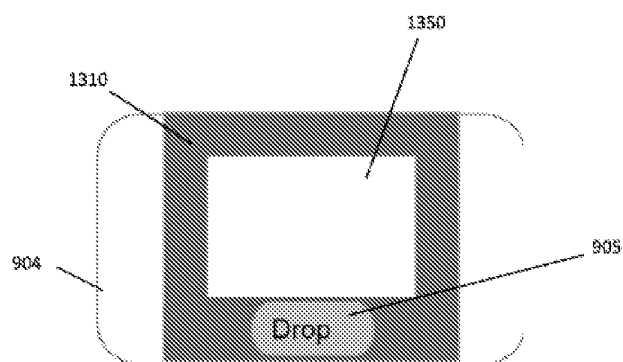


FIG. 15C

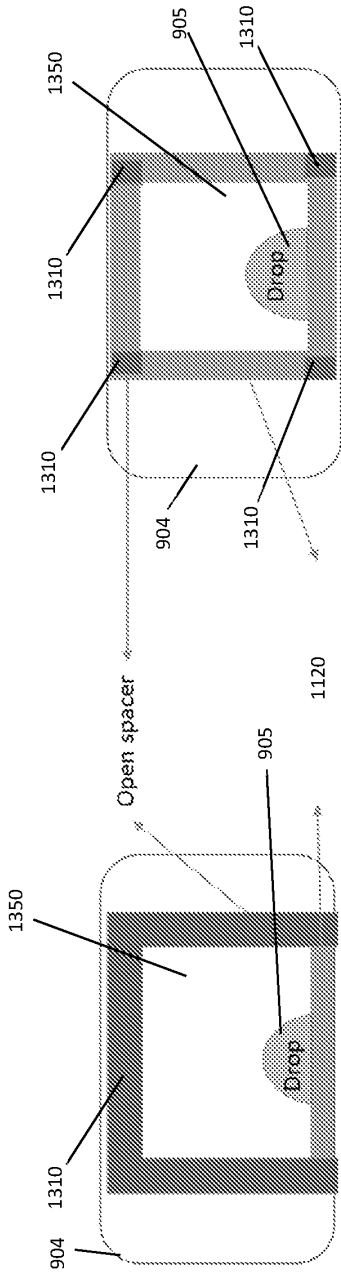


FIG. 16A

Any combination to restrict flow including partial or full enclosure is feasible
Hydrophobic pattern can be on either sides of the sandwich pair

FIG. 16B

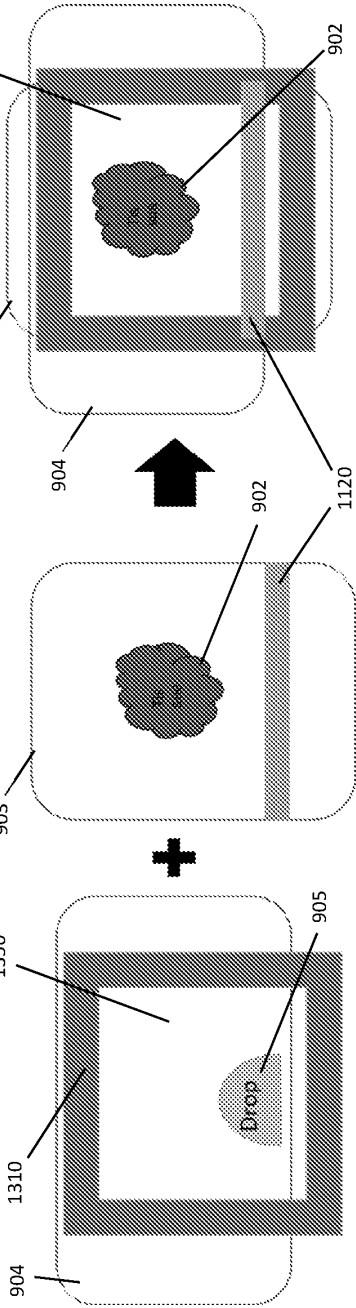


FIG. 16C

FIG. 16D

FIG. 16E

FIG. 16F

1700

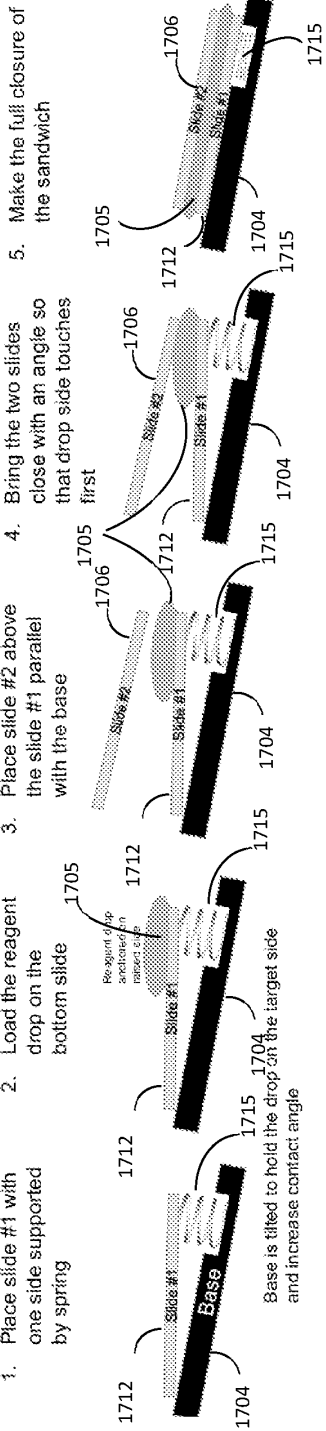


FIG. 17A

FIG. 17B

FIG. 17C

FIG. 17D

FIG. 17E

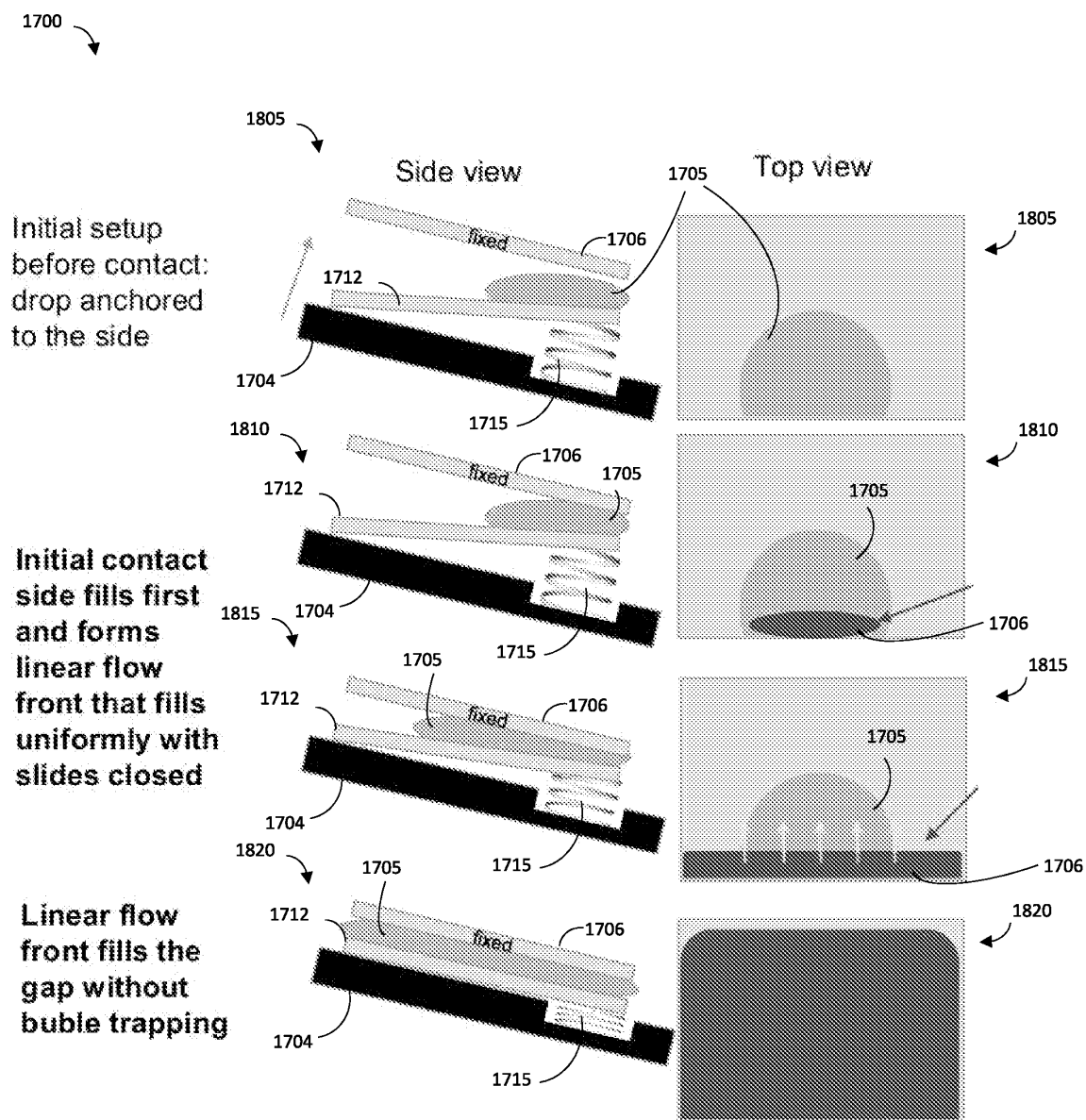


FIG. 18A

FIG. 18B

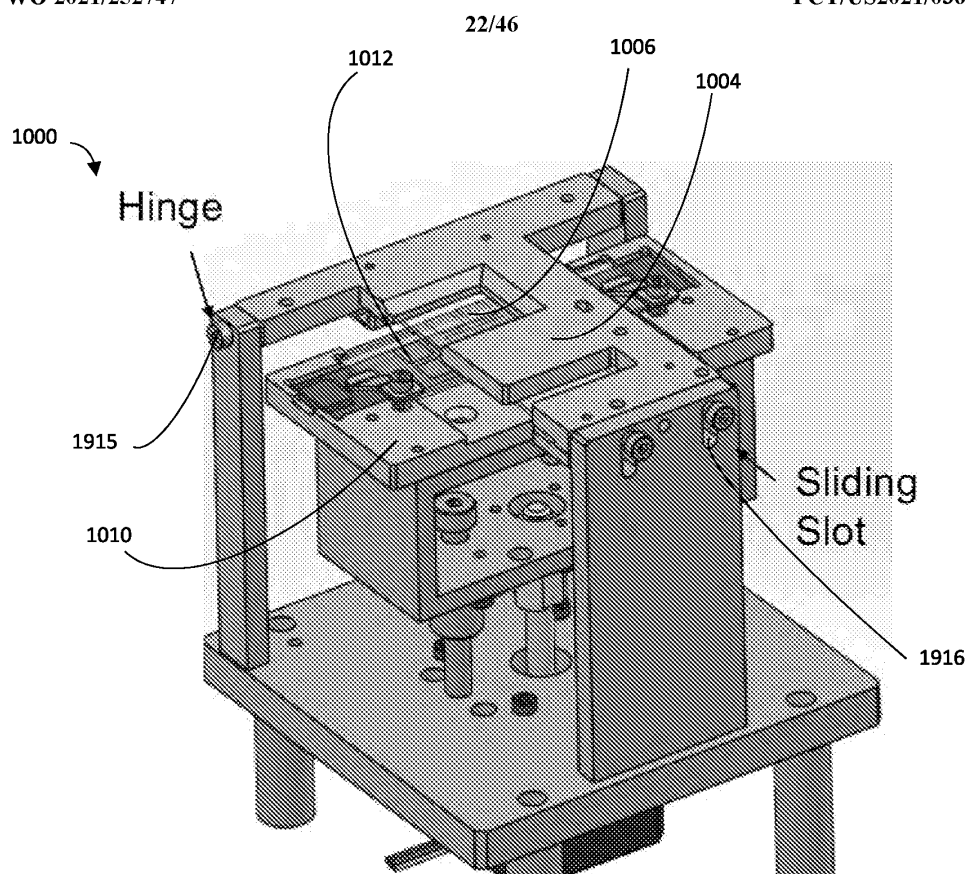


FIG. 19A

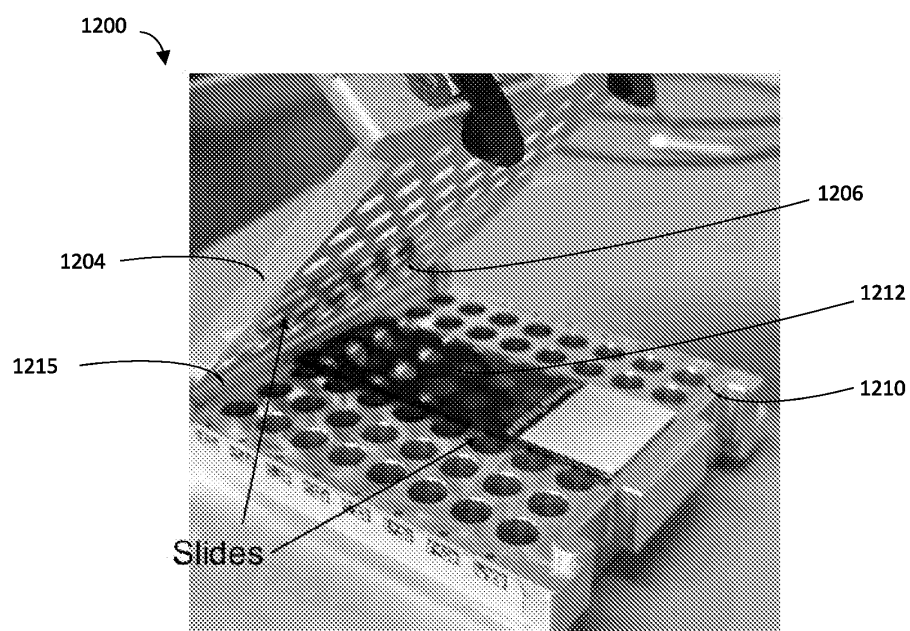
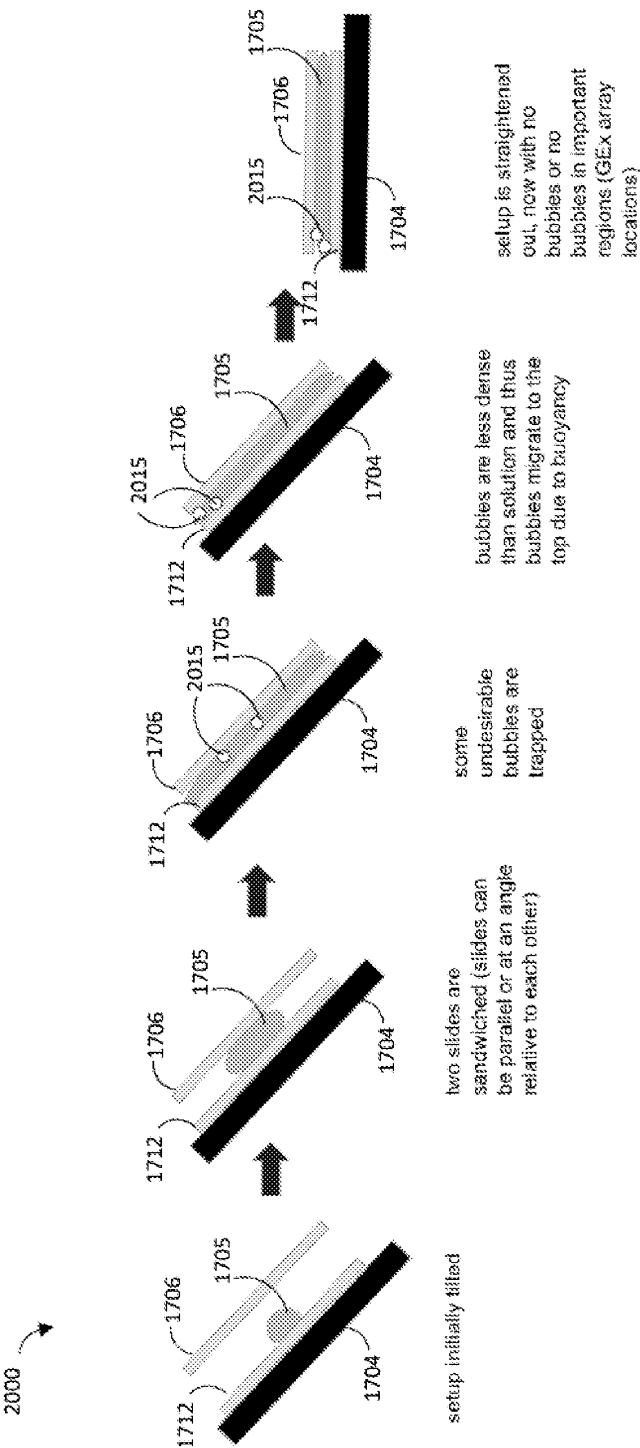
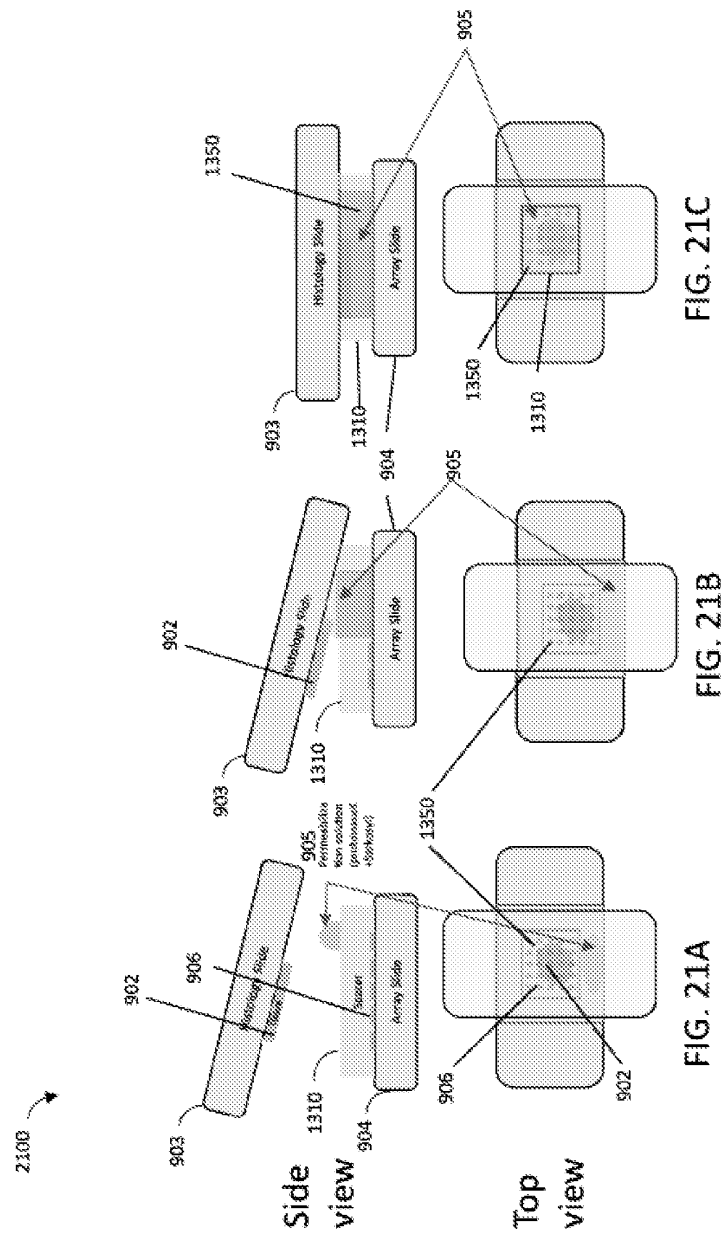


FIG. 19B





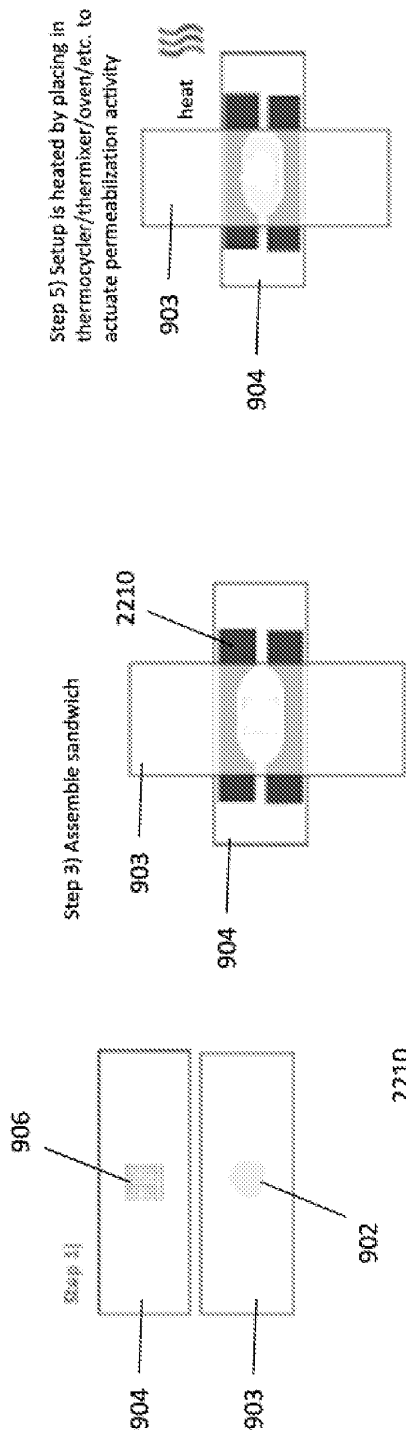
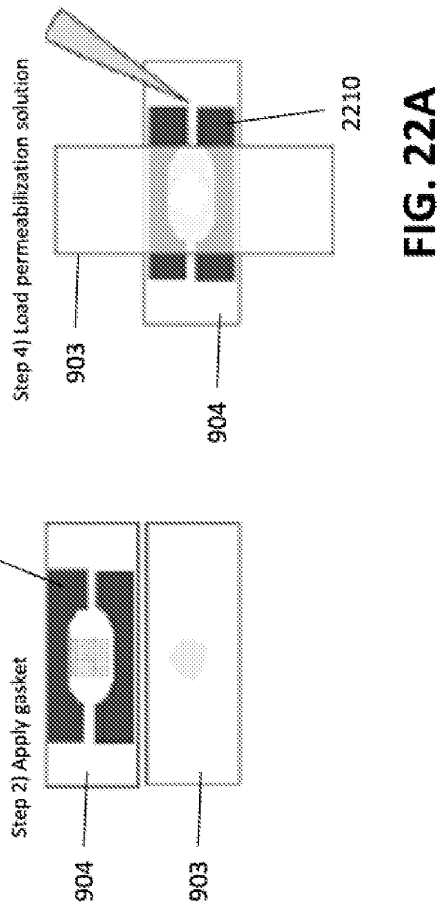


FIG. 22B



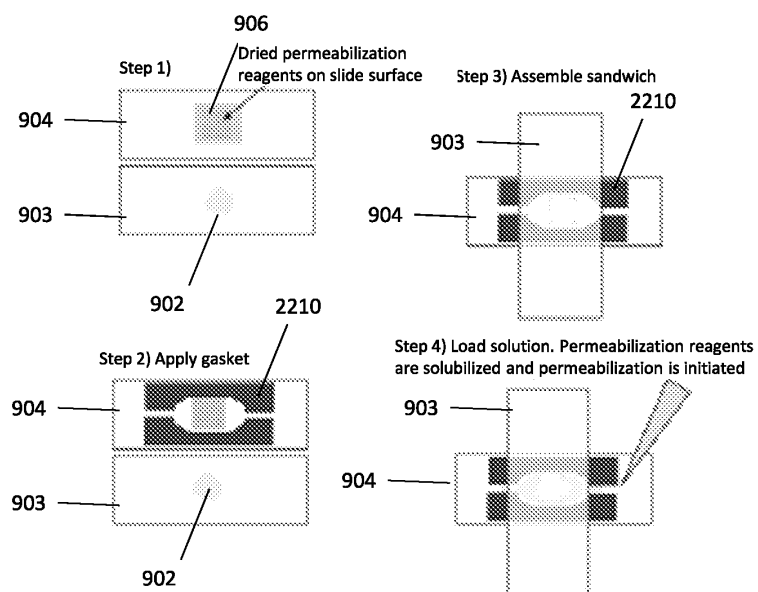


FIG. 23

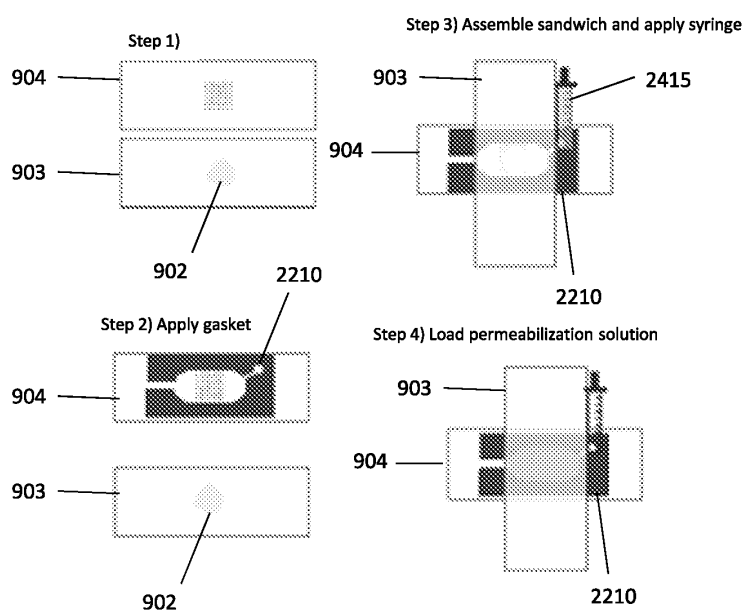


FIG. 24

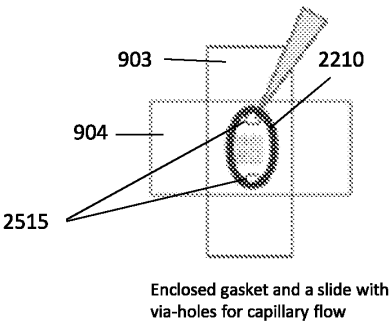


FIG. 25A

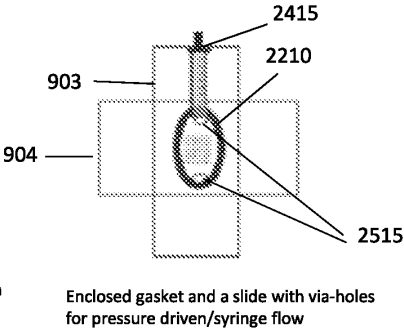
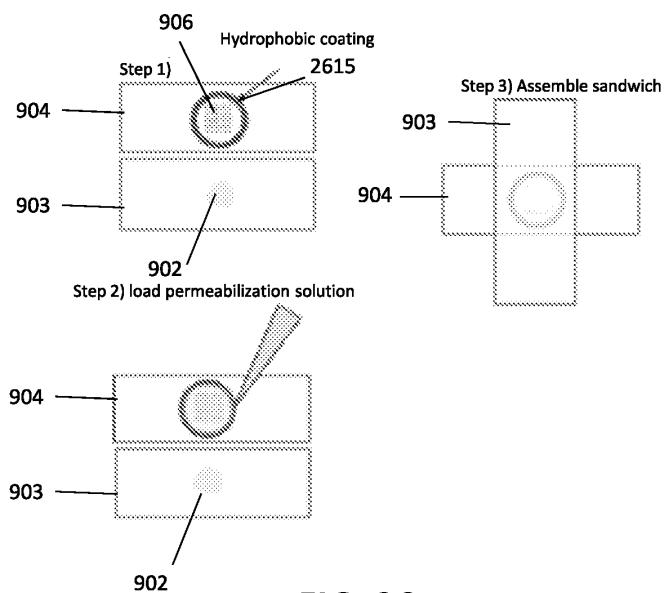


FIG. 25B

**FIG. 26**

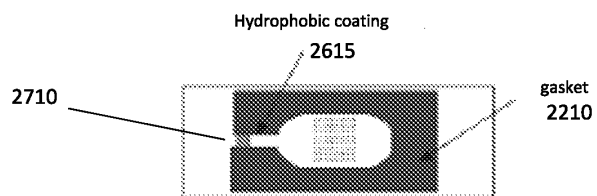
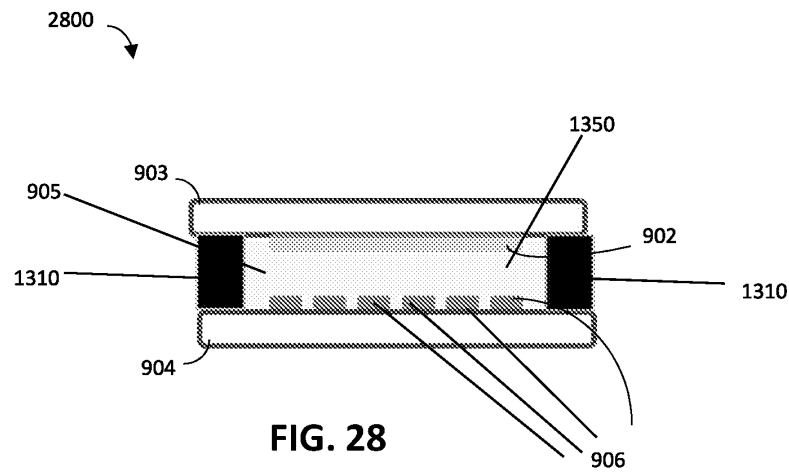


FIG. 27



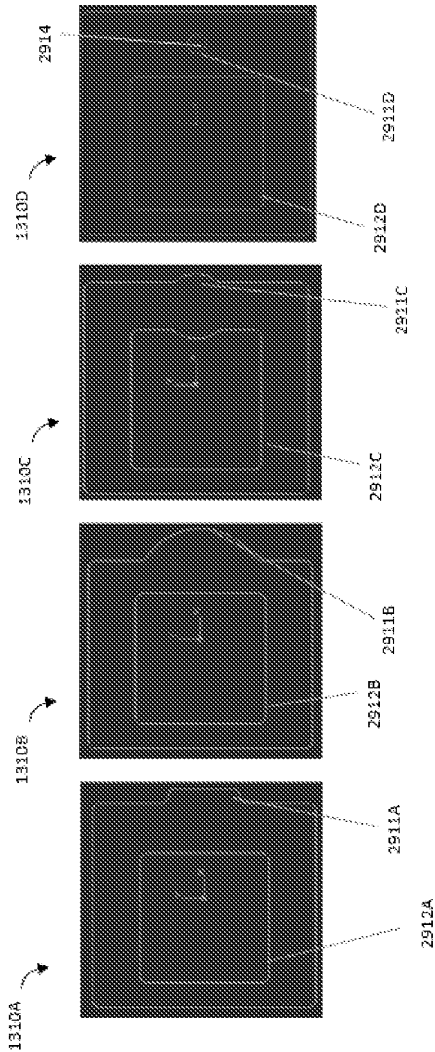


FIG. 29A

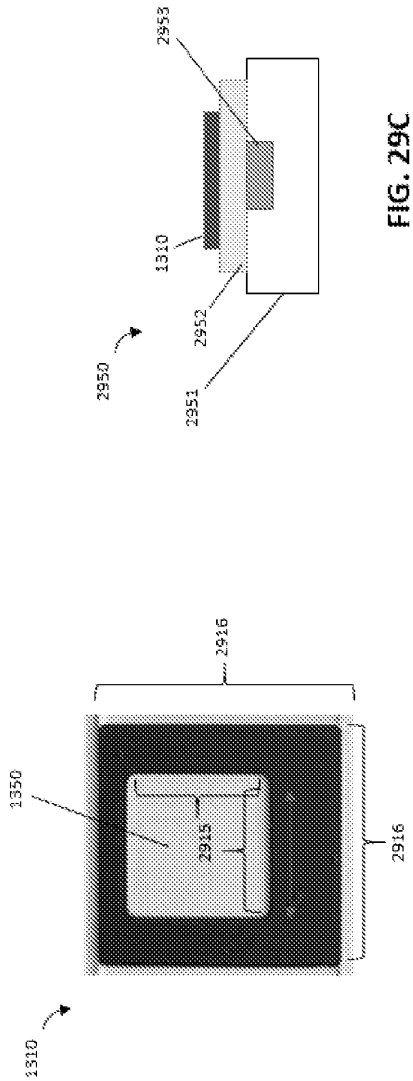


FIG. 29B

FIG. 29C

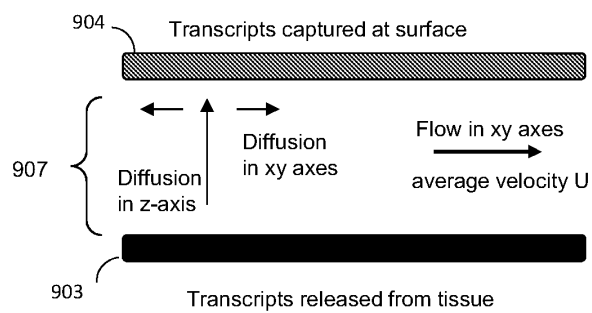
3000 

FIG. 30

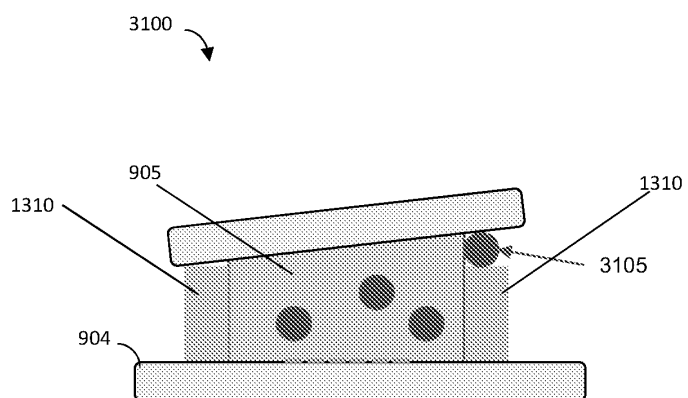


FIG. 31A

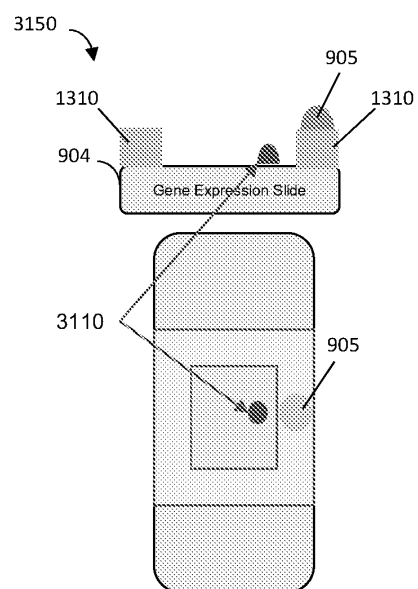


FIG. 31B

3200 ↘

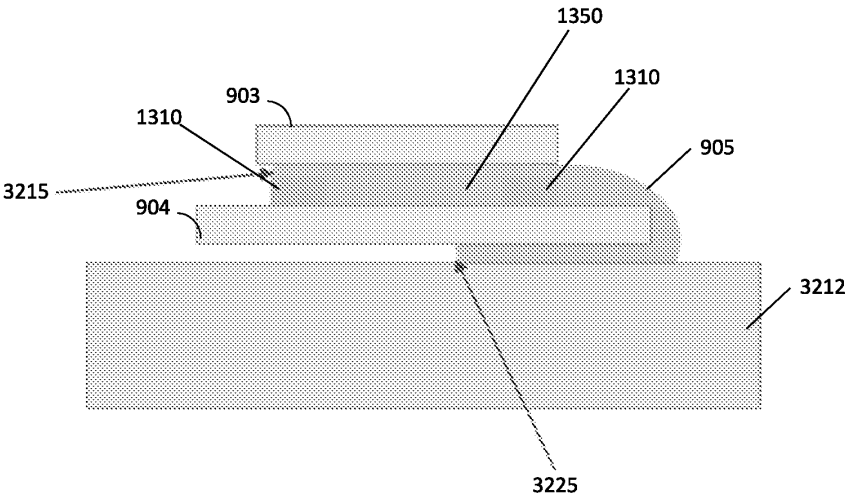


FIG. 32A

3250 ↘

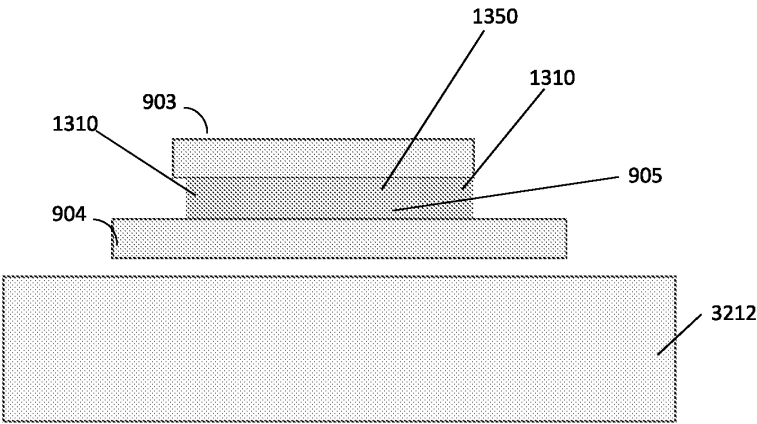


FIG. 32B

3300 ↘

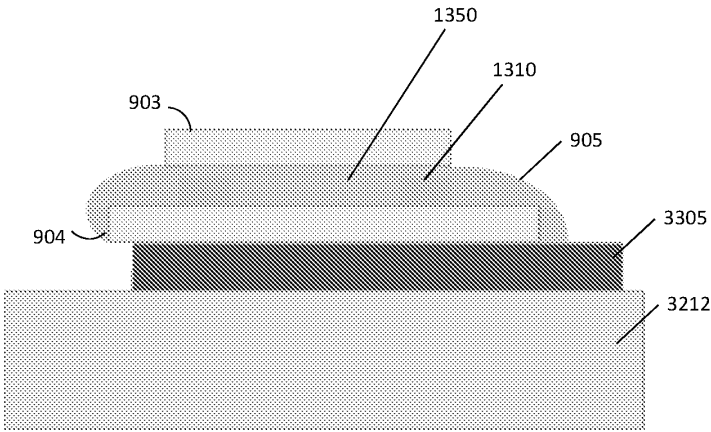


FIG. 33

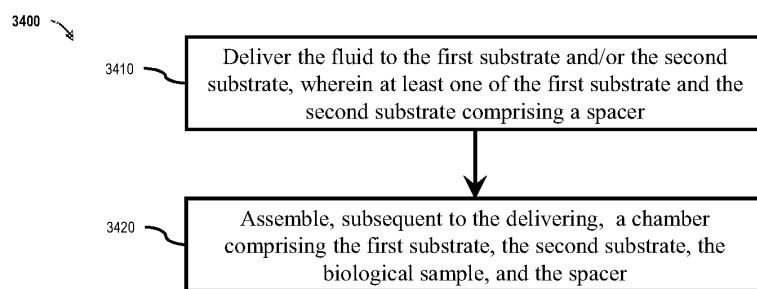


FIG. 34

4400

Analysis ID	Sample	Description	Permeabilization time	Permeabilization buffer	mm10 Median genes per spot (30k raw reads per spot)	mm10 Median umi counts per spot (30k raw reads per spot)
1046321	Mouse Brains (FF)	Non-sandwich	5 minutes	30mM Tris (pH7.4); 5% Sarkosyl	5194	17660
1046322		Permeabilization in sandwich assembly	1 minute		4592	13083
1046514					4072	14017
1046515					3758	13731

FIG. 35

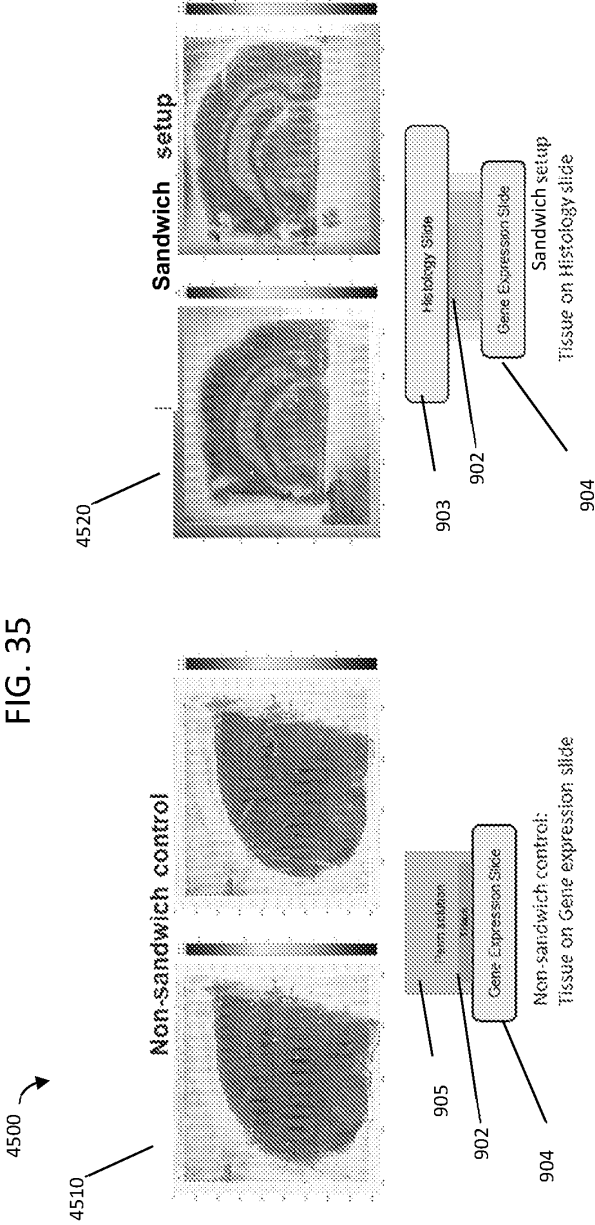


FIG. 36

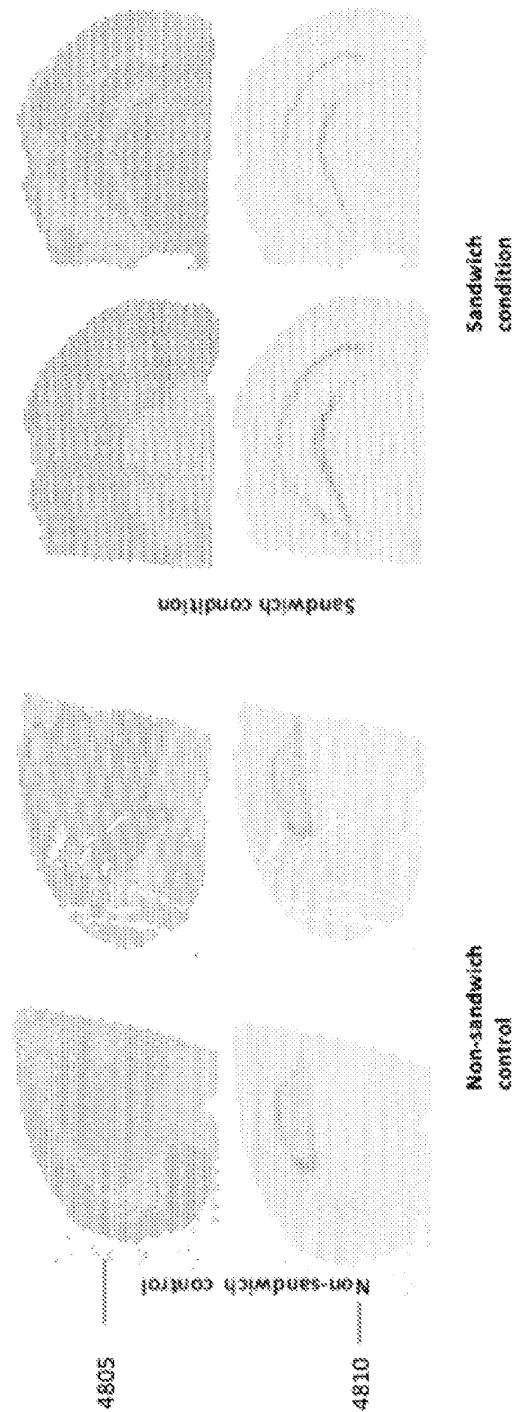


FIG. 37

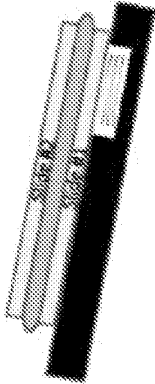
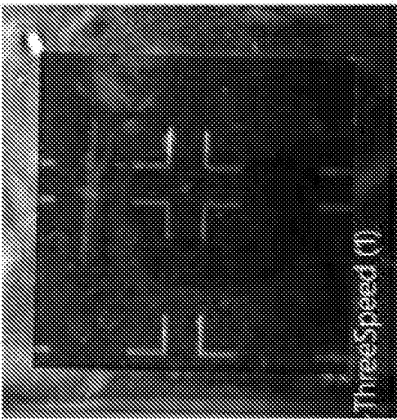


FIG. 38C

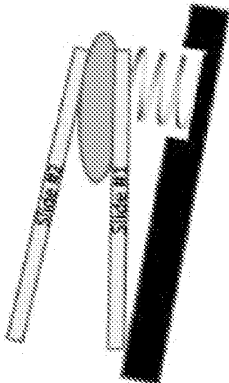
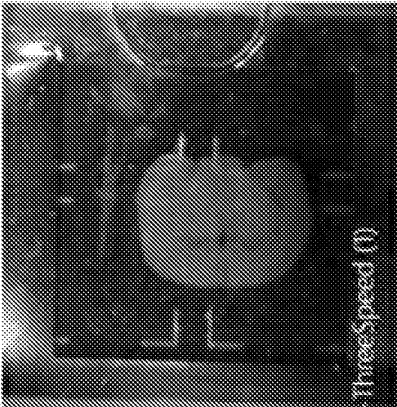


FIG. 38B

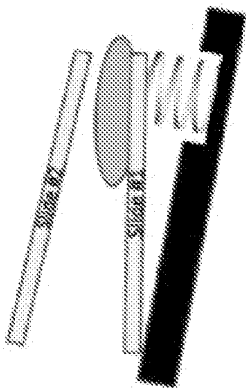
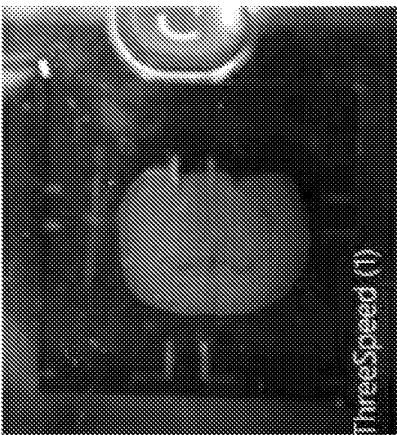


FIG. 38A

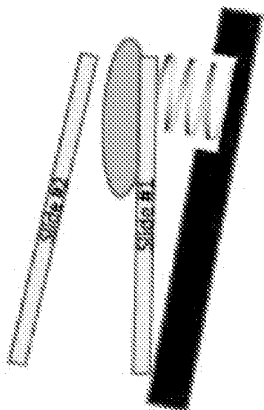
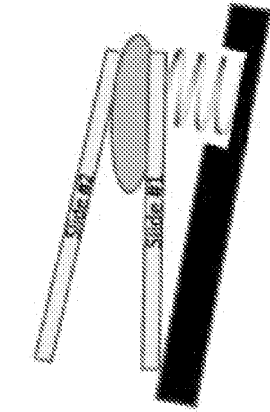
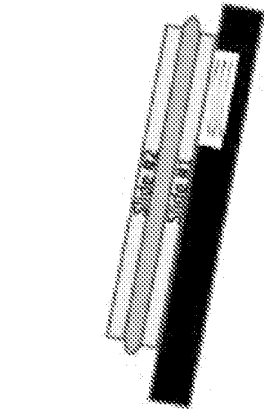
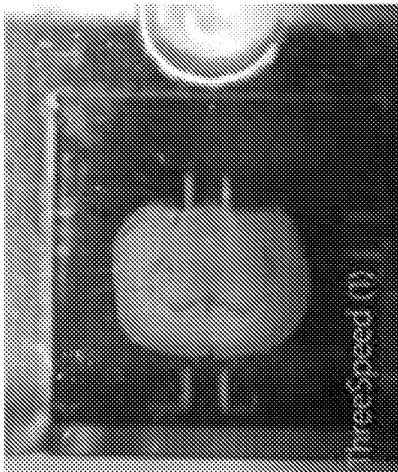
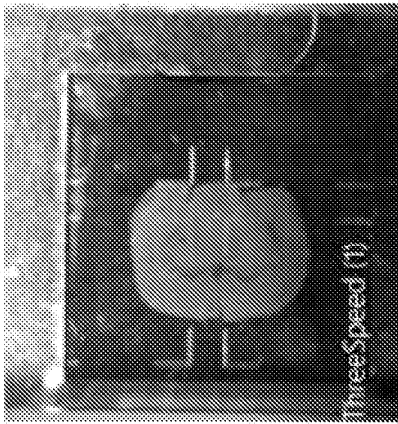
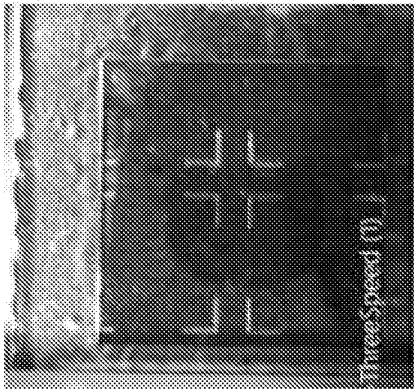


FIG. 39C

FIG. 39B

FIG. 39A

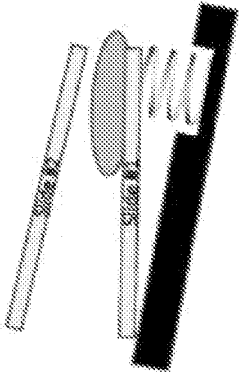
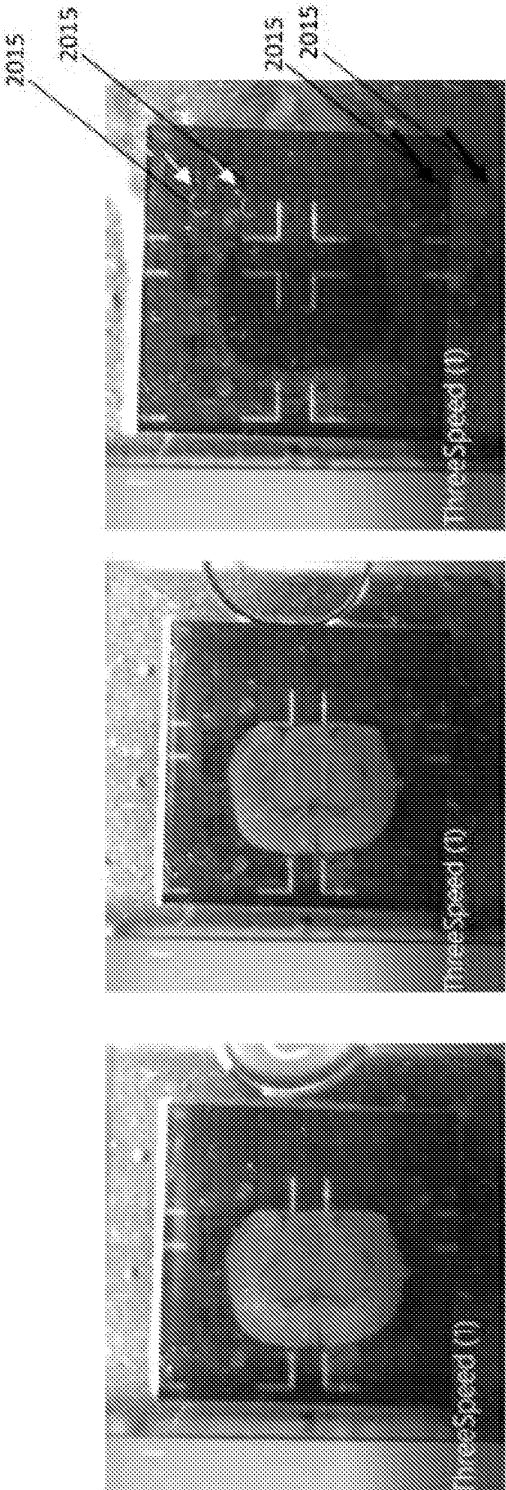


FIG. 40A

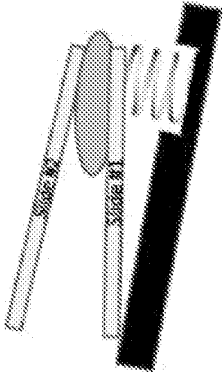


FIG. 40B

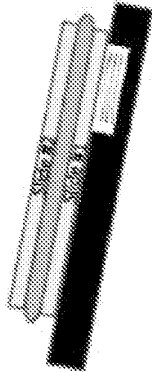


FIG. 40C

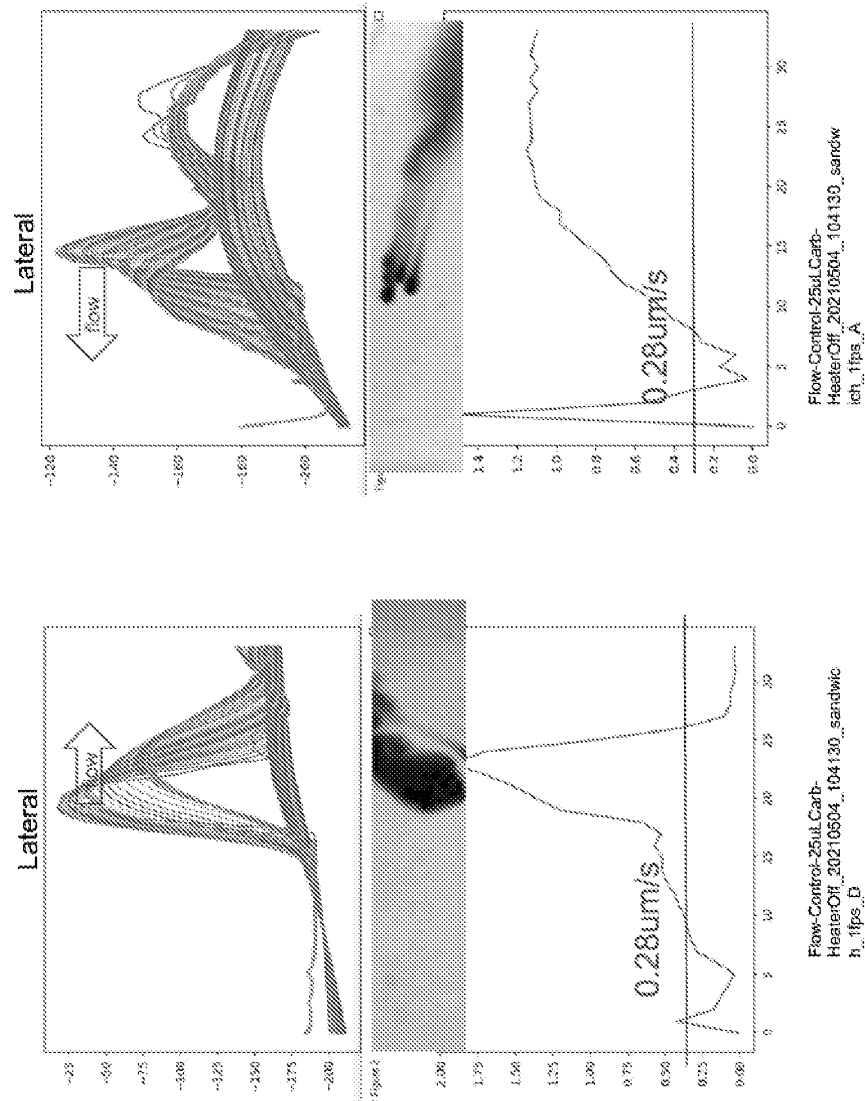


FIG. 41B

FIG. 41A

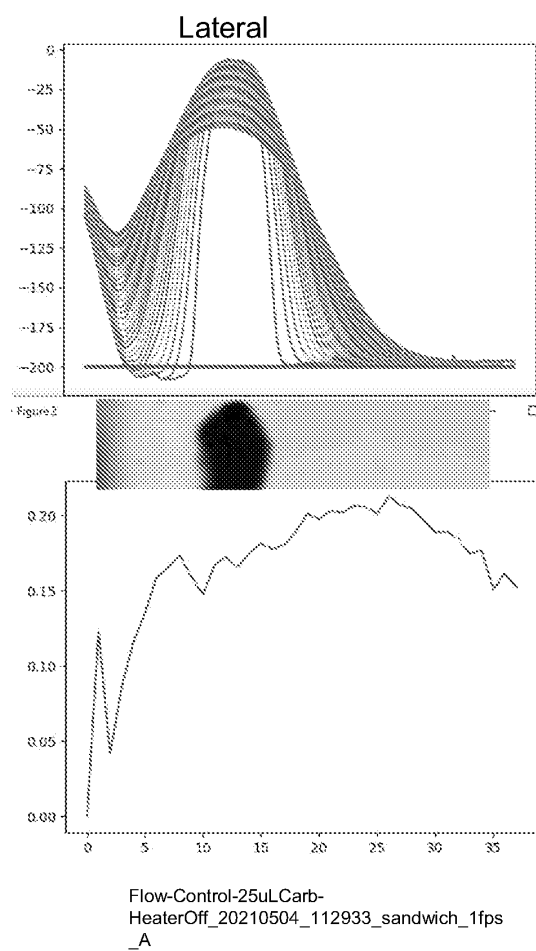


FIG. 41C

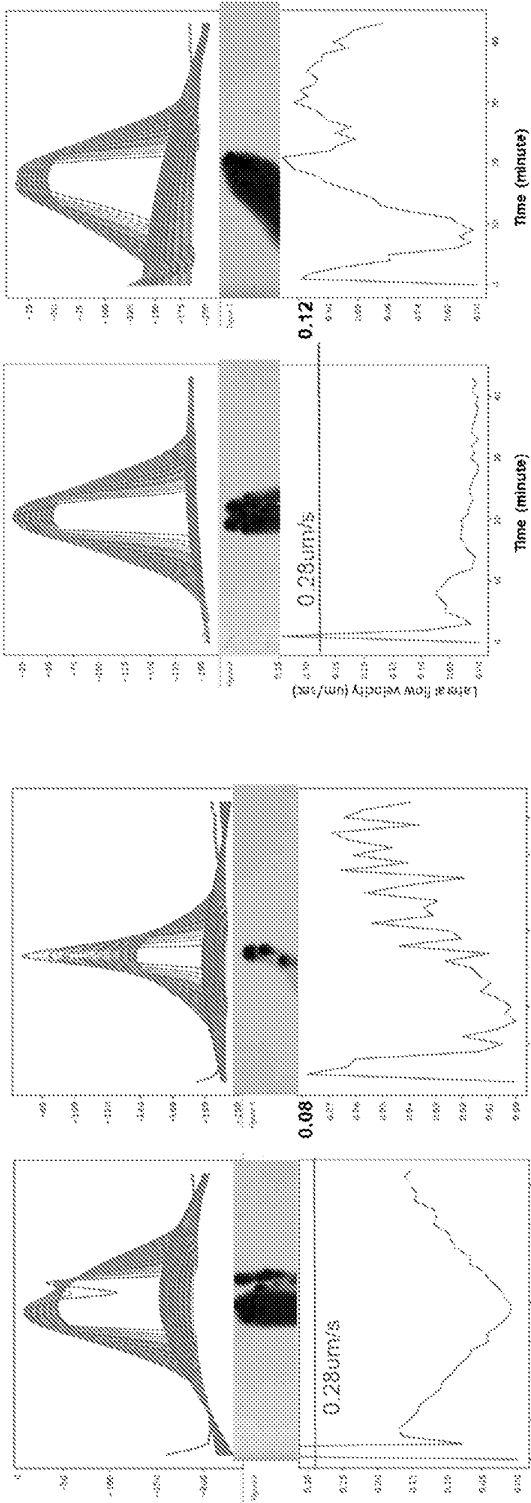


FIG. 42D

FIG. 42C

FIG. 42B

FIG. 42A

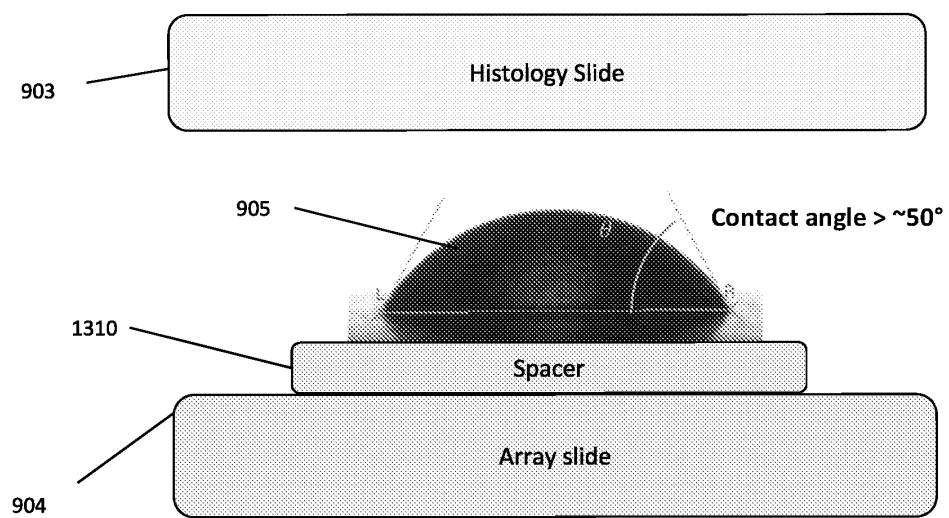


FIG. 43