

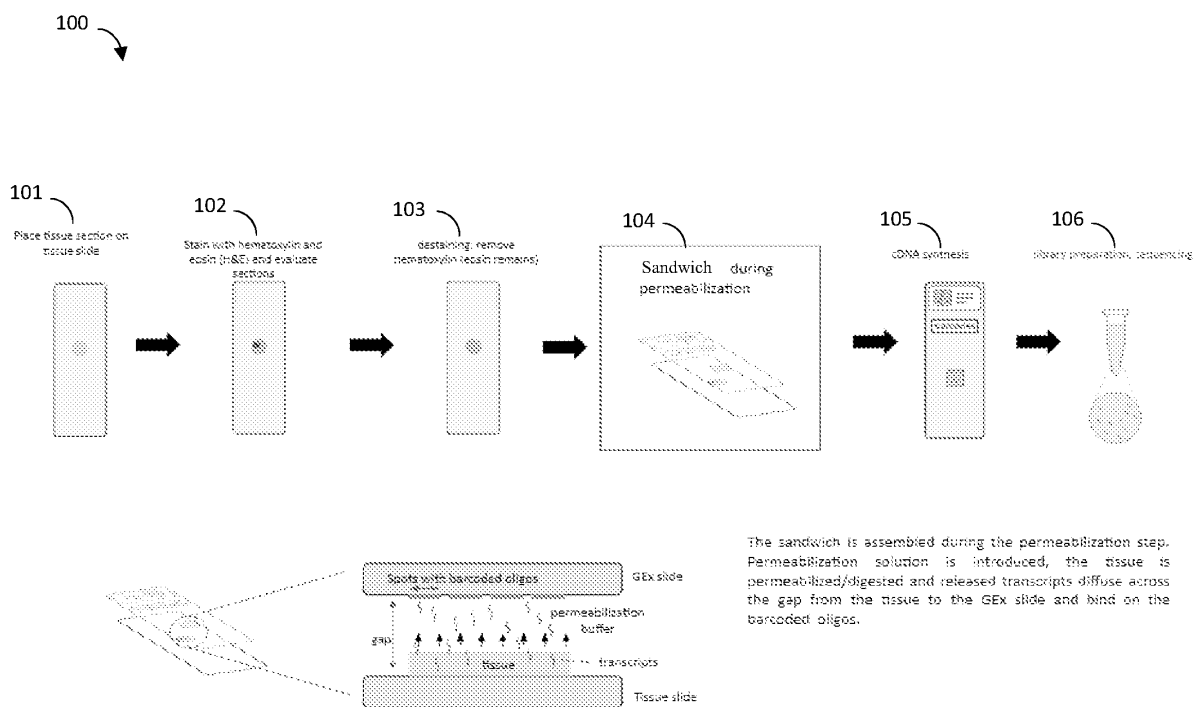


FIG. 1

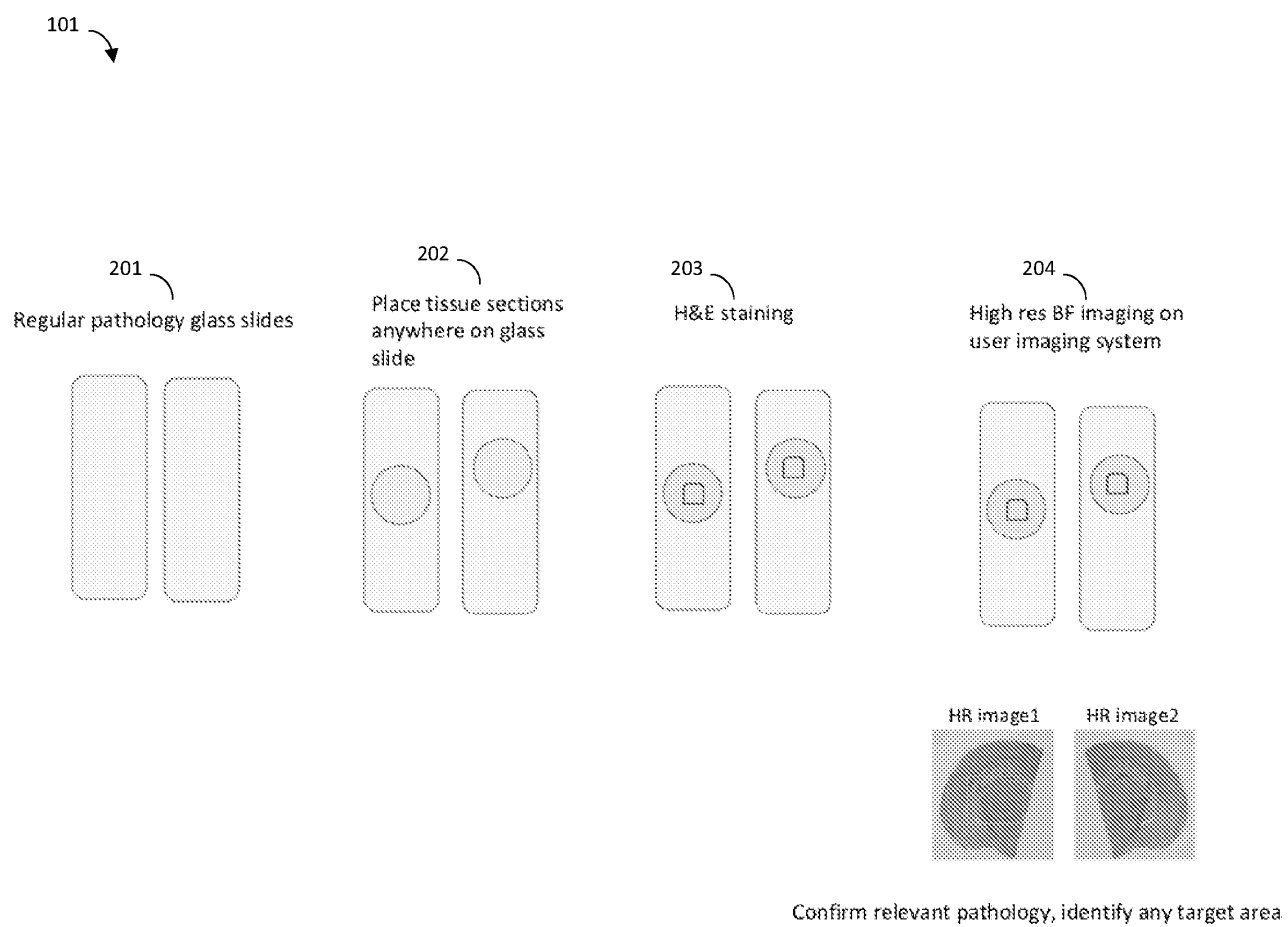


FIG. 2

104

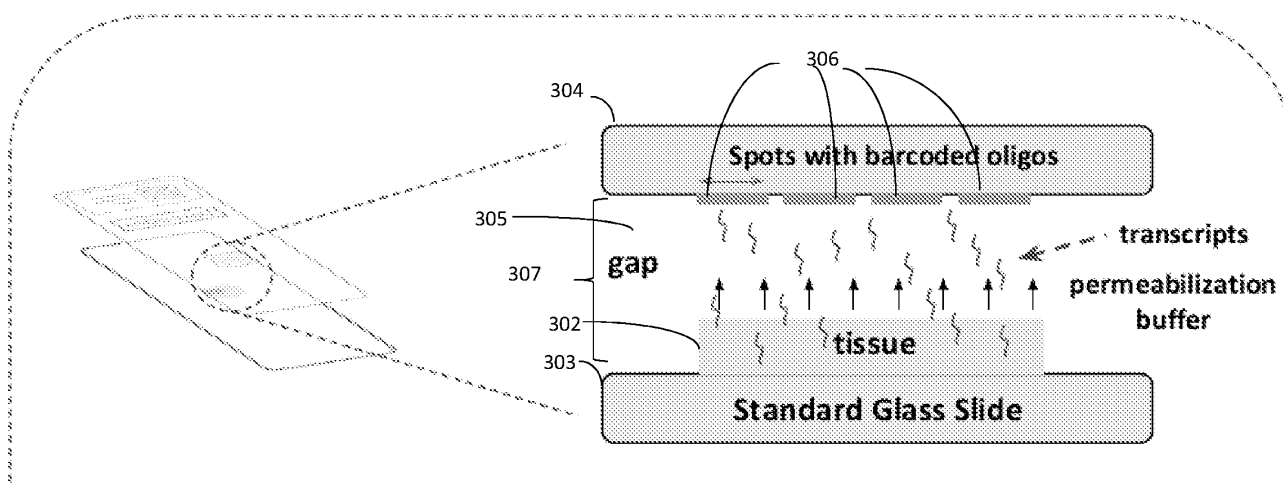


FIG. 3

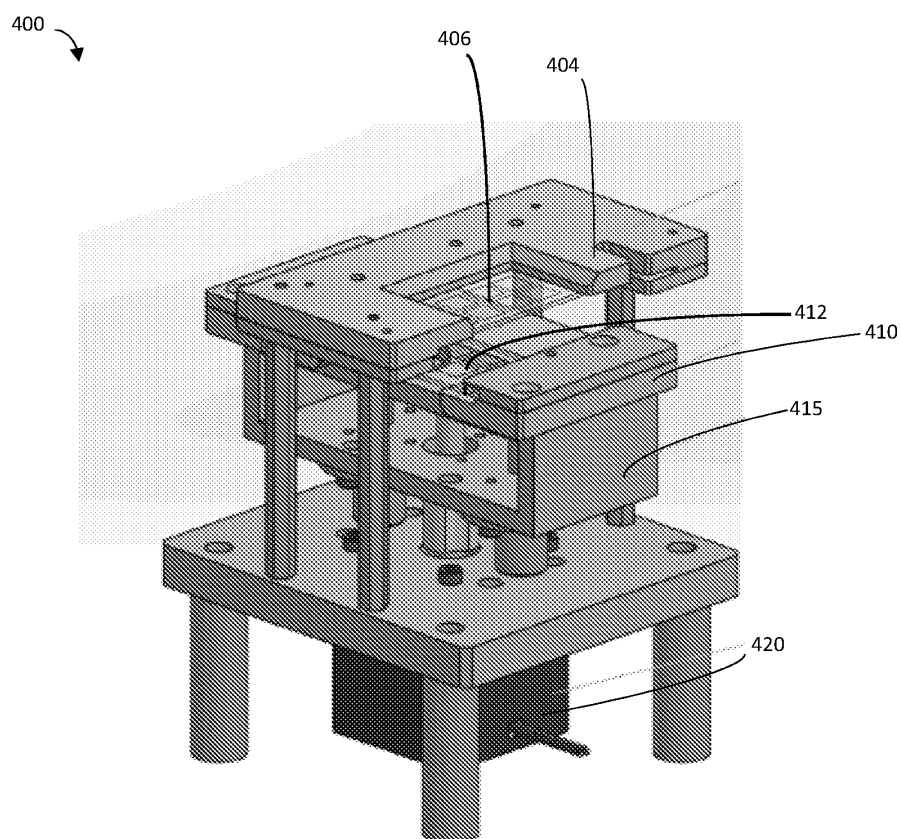


FIG. 4

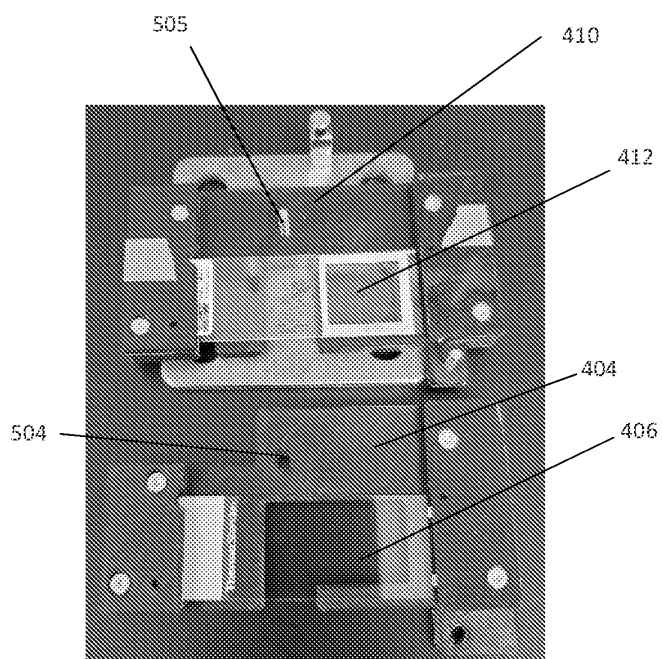


FIG. 5A

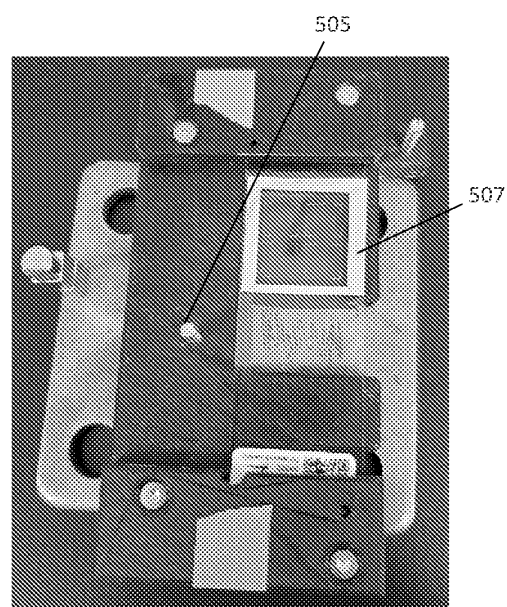


FIG. 5B

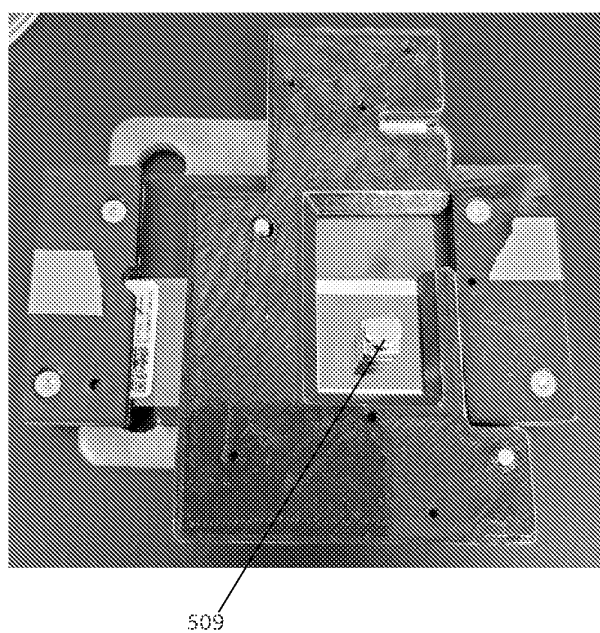


FIG. 5C

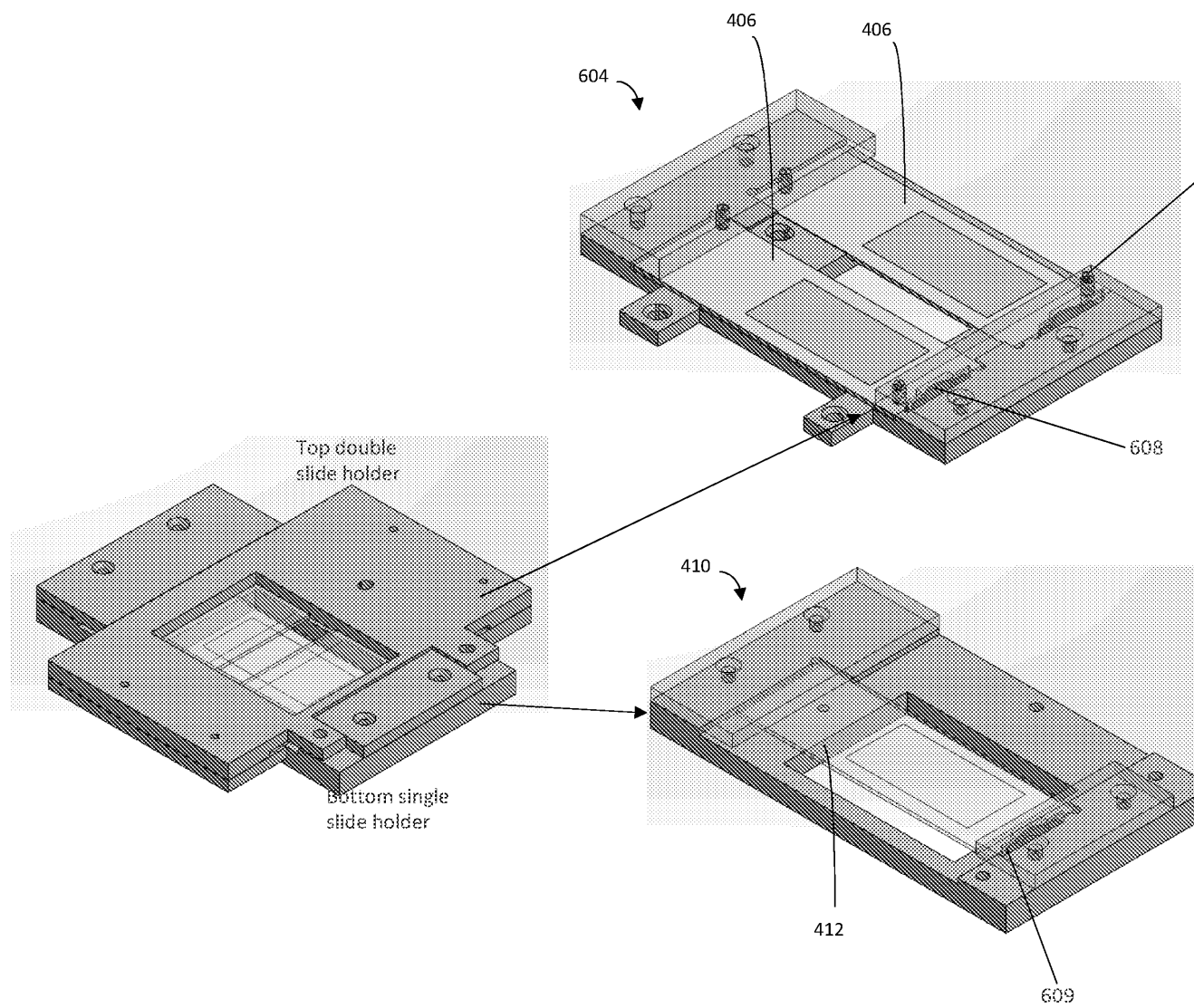


FIG. 6

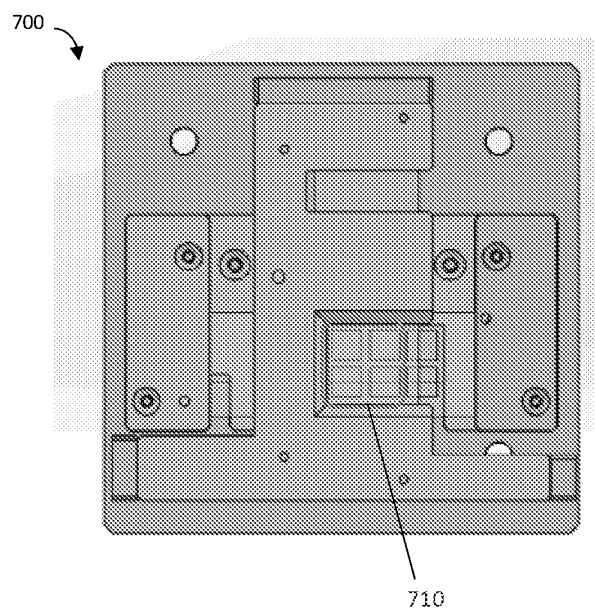
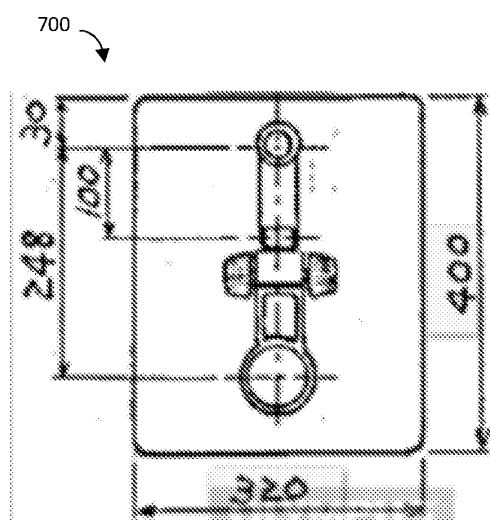


FIG. 7

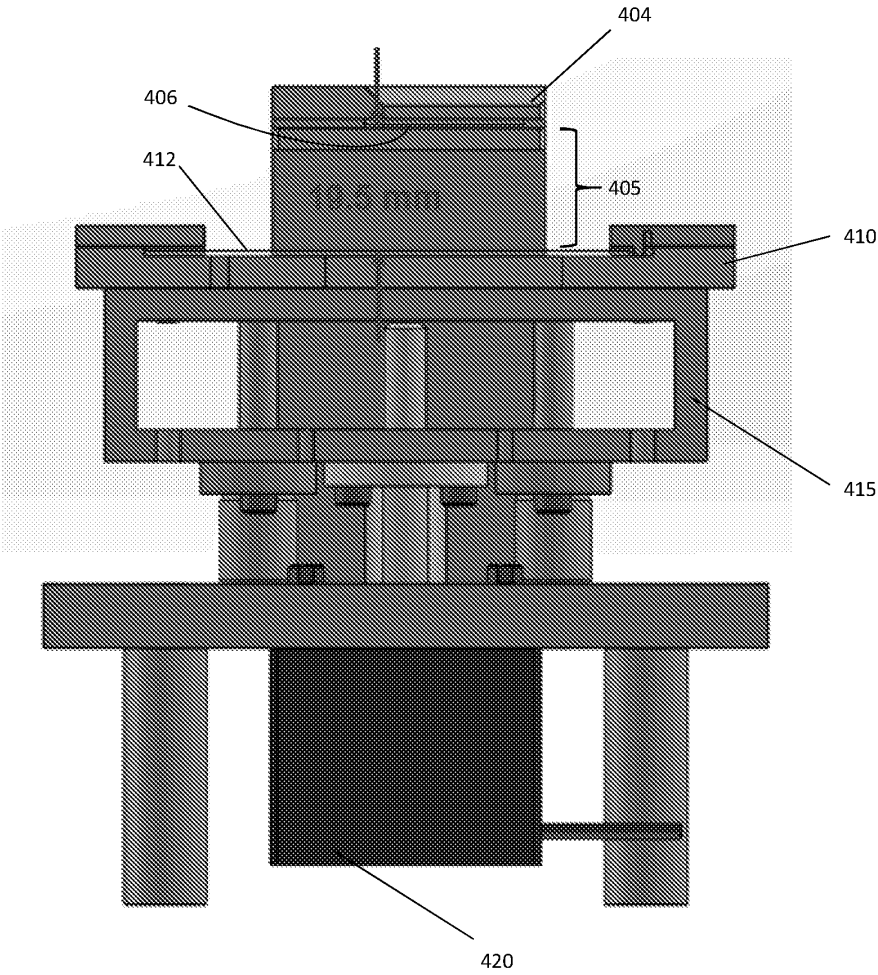


FIG. 8

415

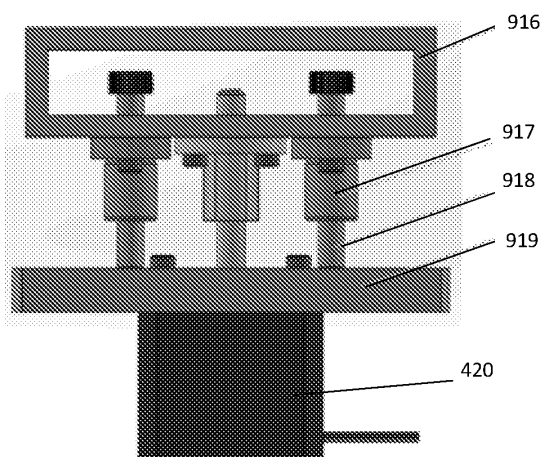


FIG. 9

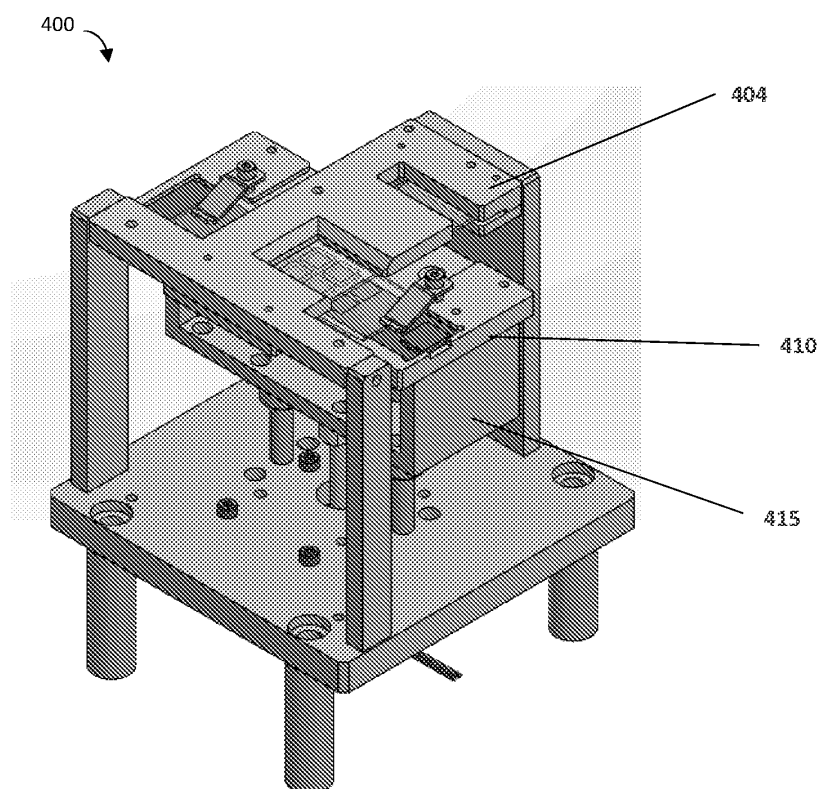


FIG. 10

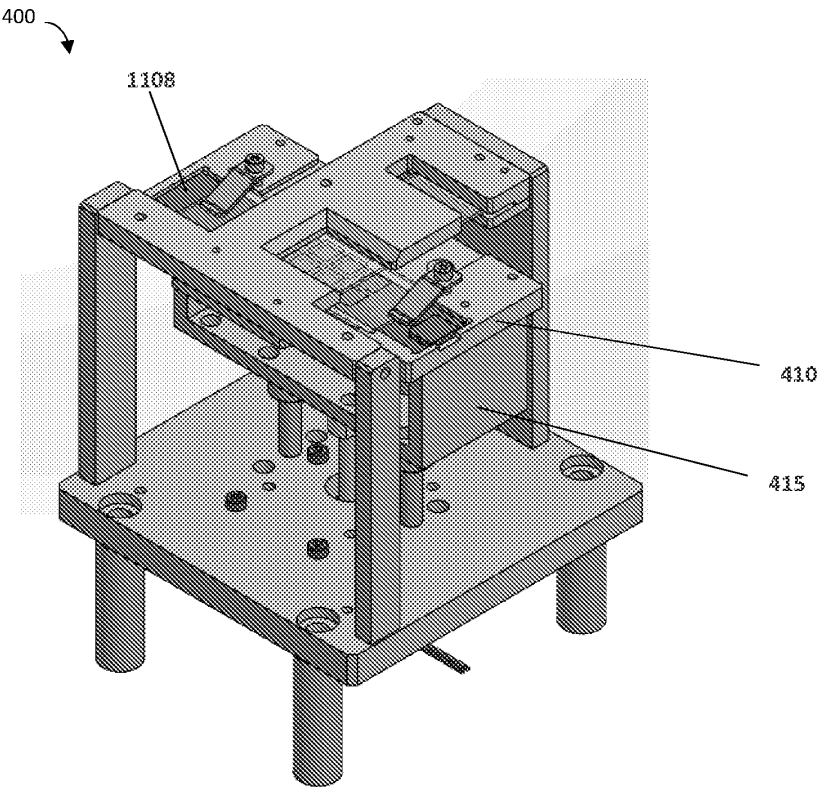


FIG. 11A

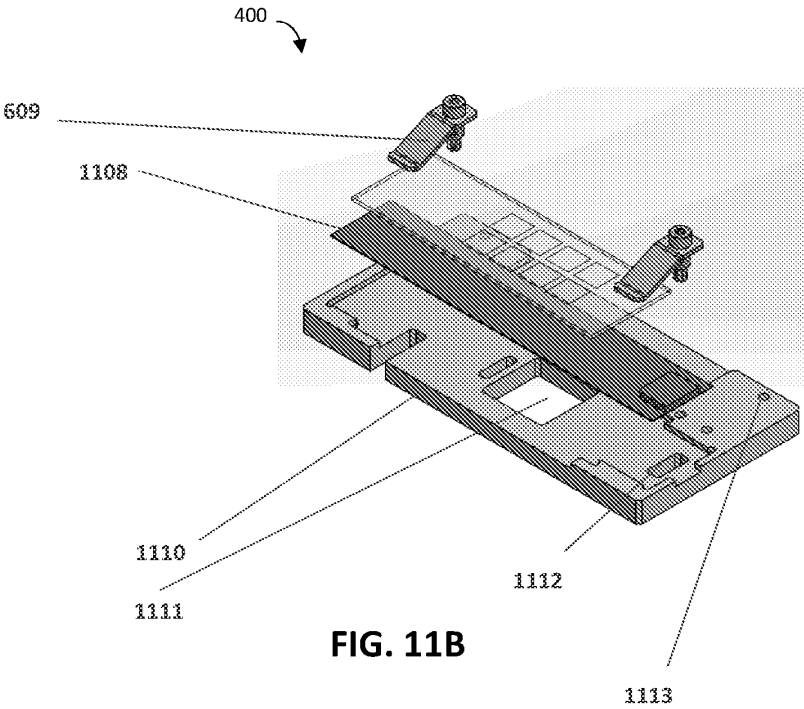


FIG. 11B

1150

Desired Slide Temperature Profile

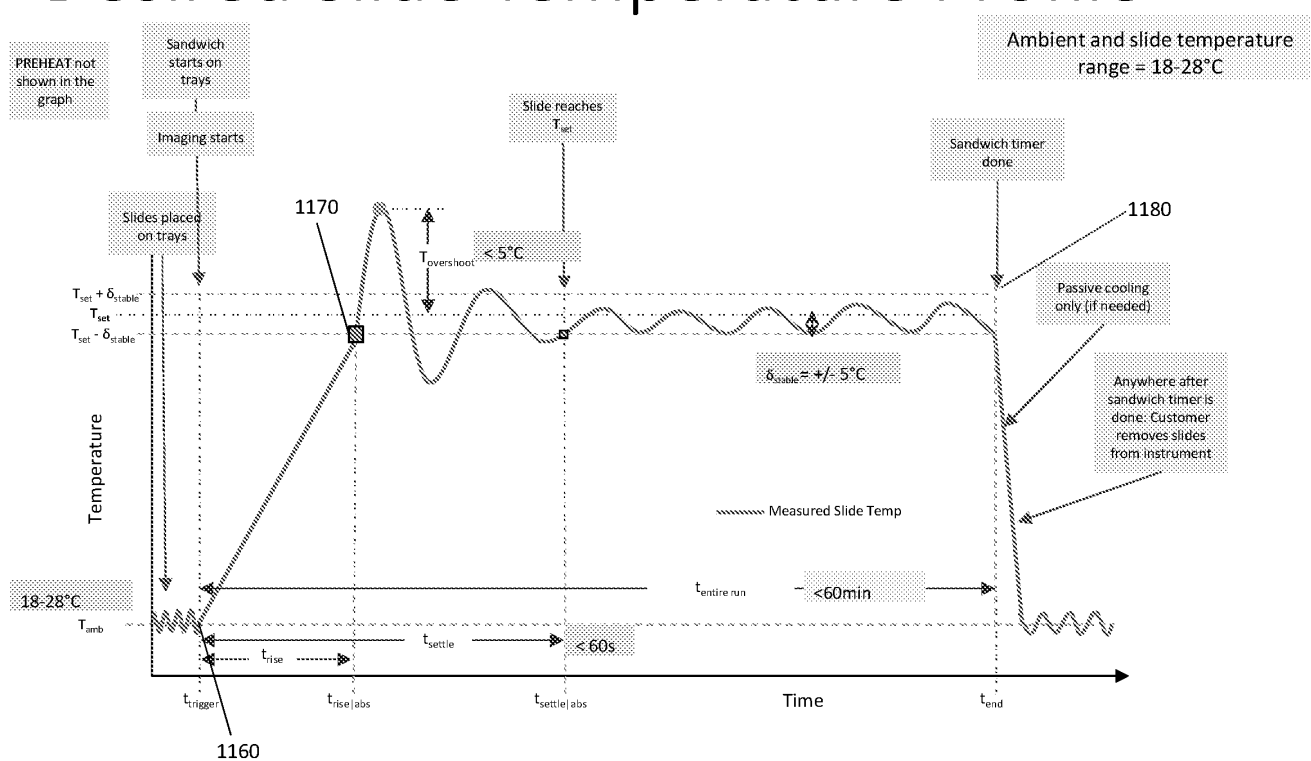


FIG. 11C

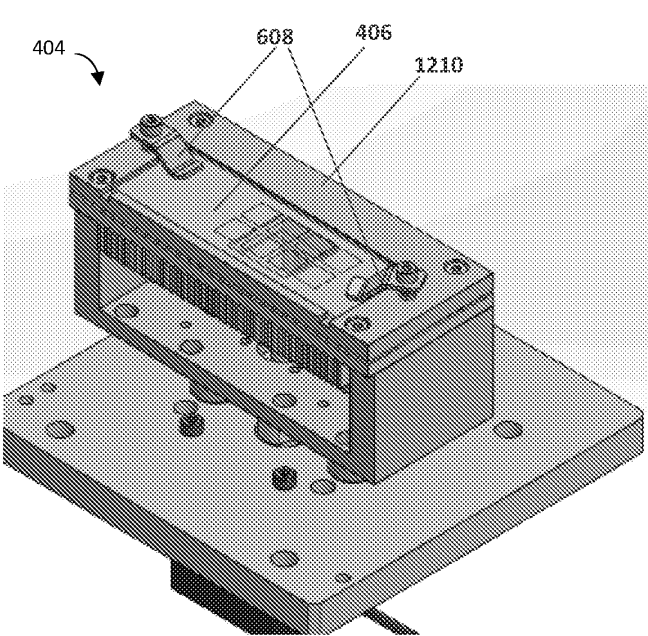


FIG. 12A

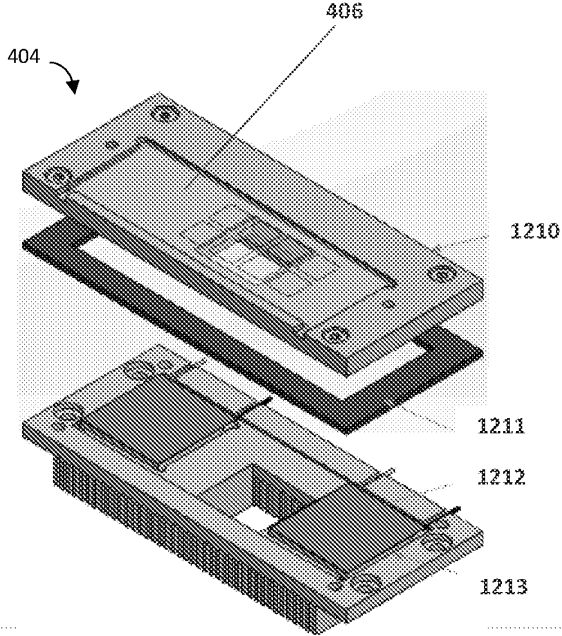


FIG. 12B

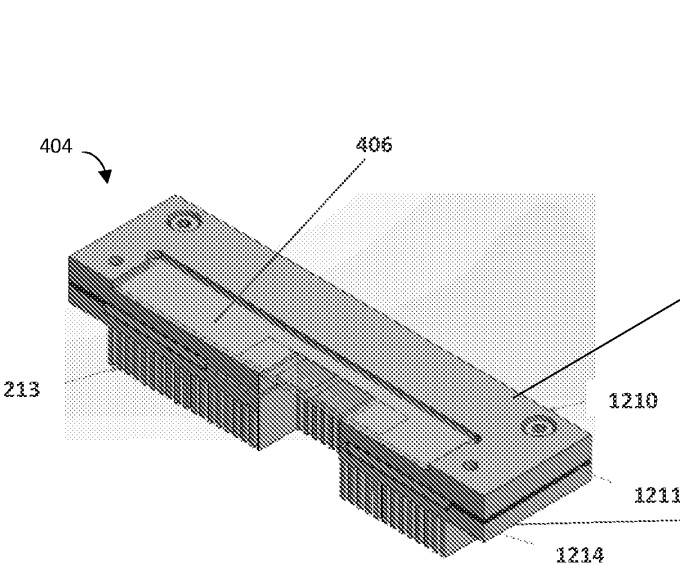


FIG. 13A

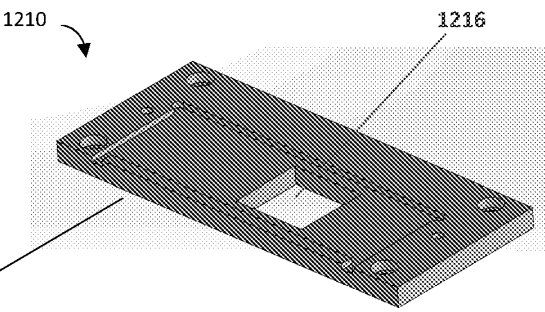


FIG. 13B

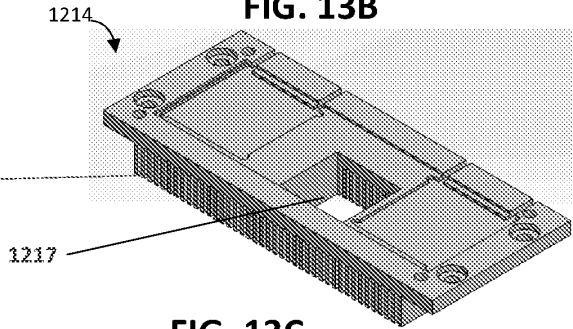


FIG. 13C

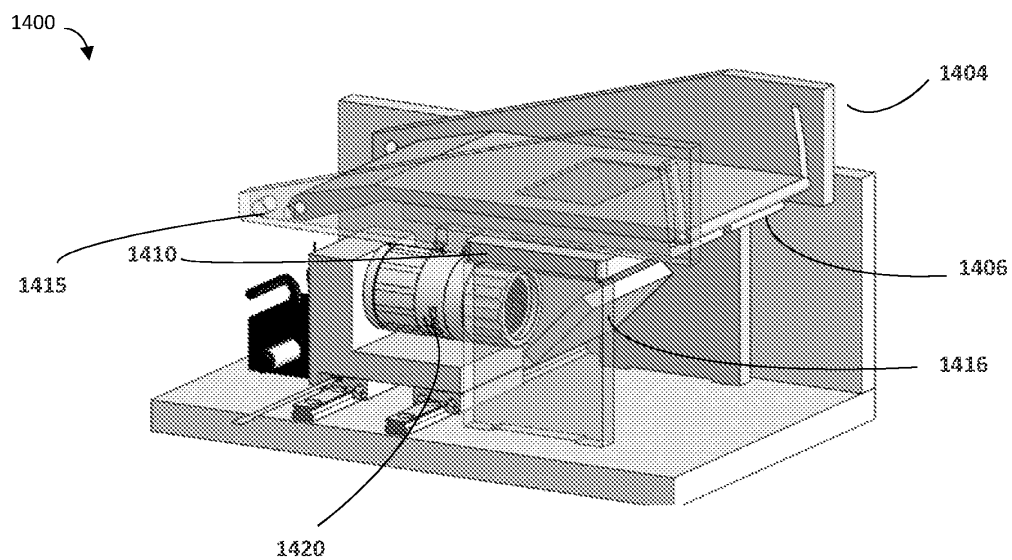


FIG. 14A

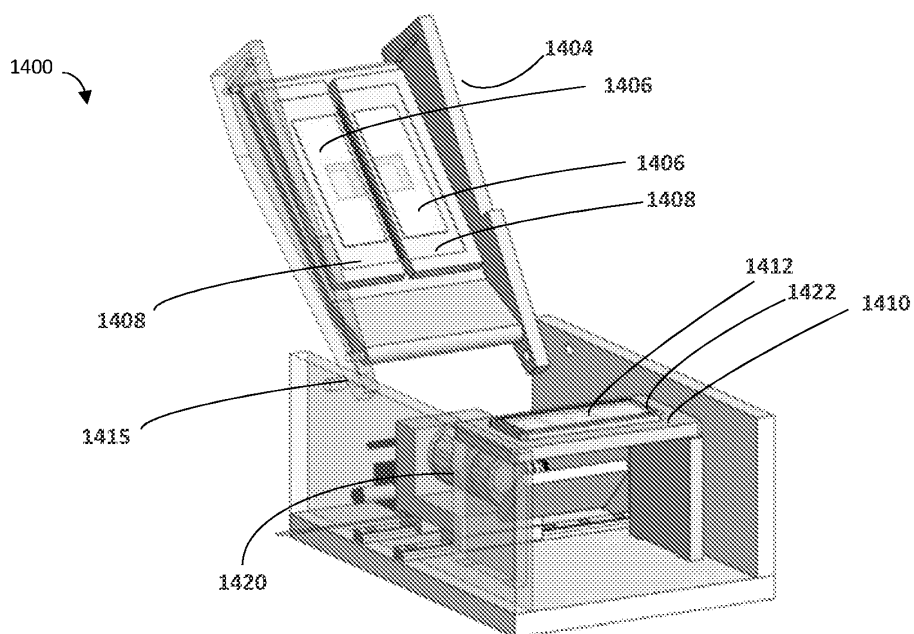


FIG. 14B

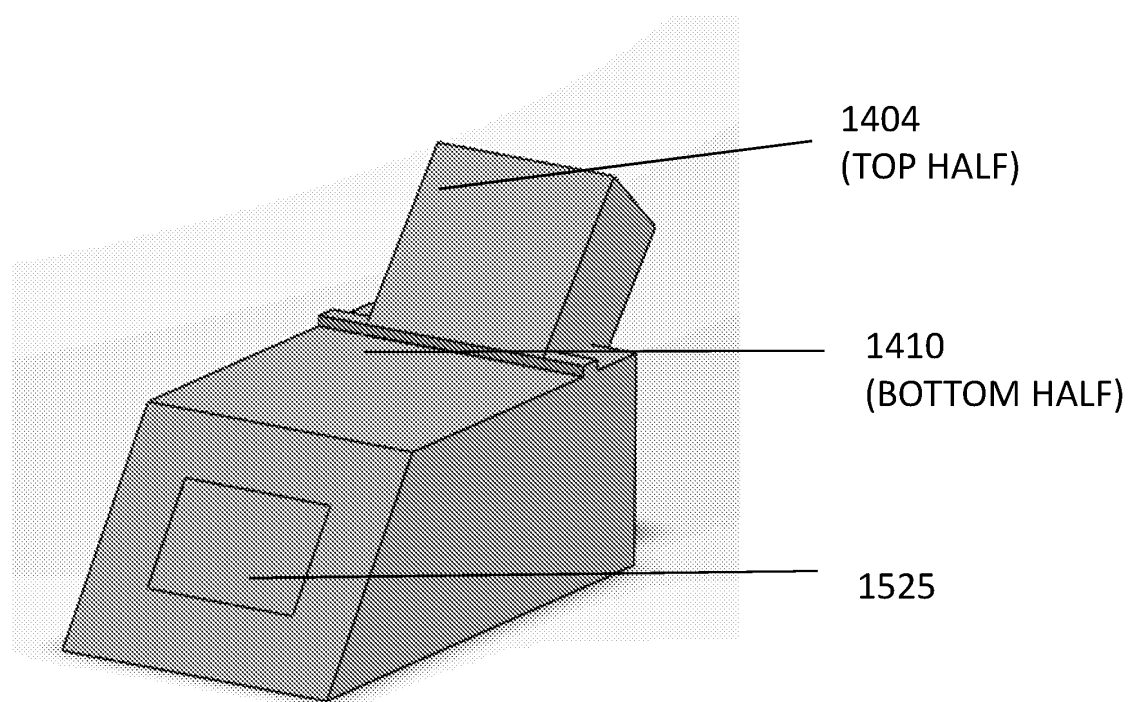


FIG. 15

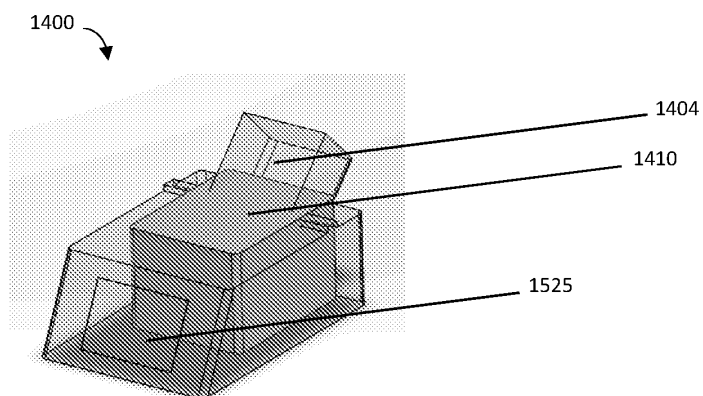


FIG. 16A

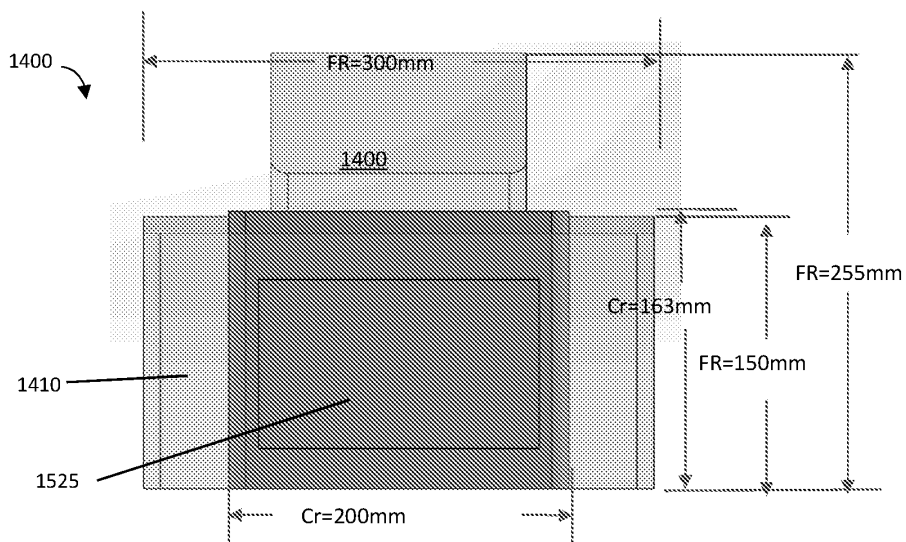


FIG. 16B

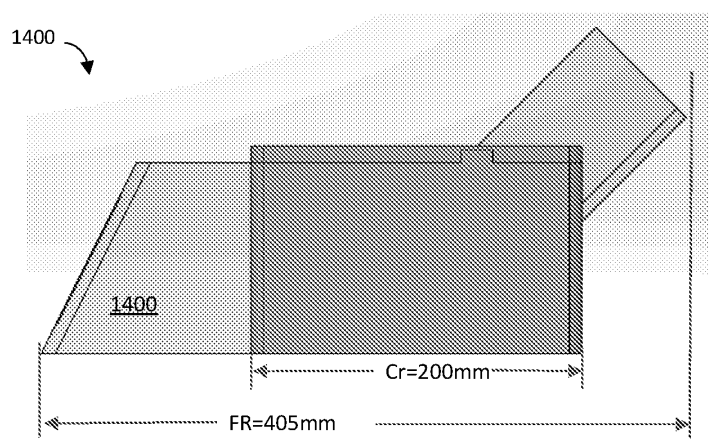


FIG. 16C

1700

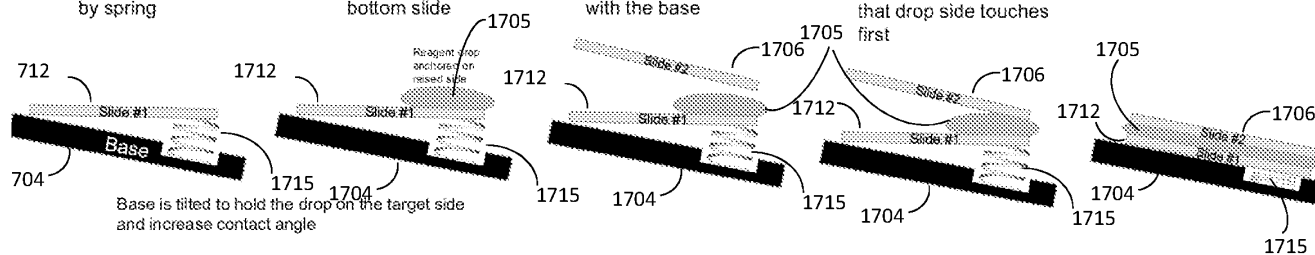
1. Place slide #1 with one side supported by spring

2. Load the reagent drop on the bottom slide

3. Place slide #2 above the slide #1 parallel with the base

4. Bring the two slides close with an angle so that drop side touches first

5. Make the full closure of the sandwich



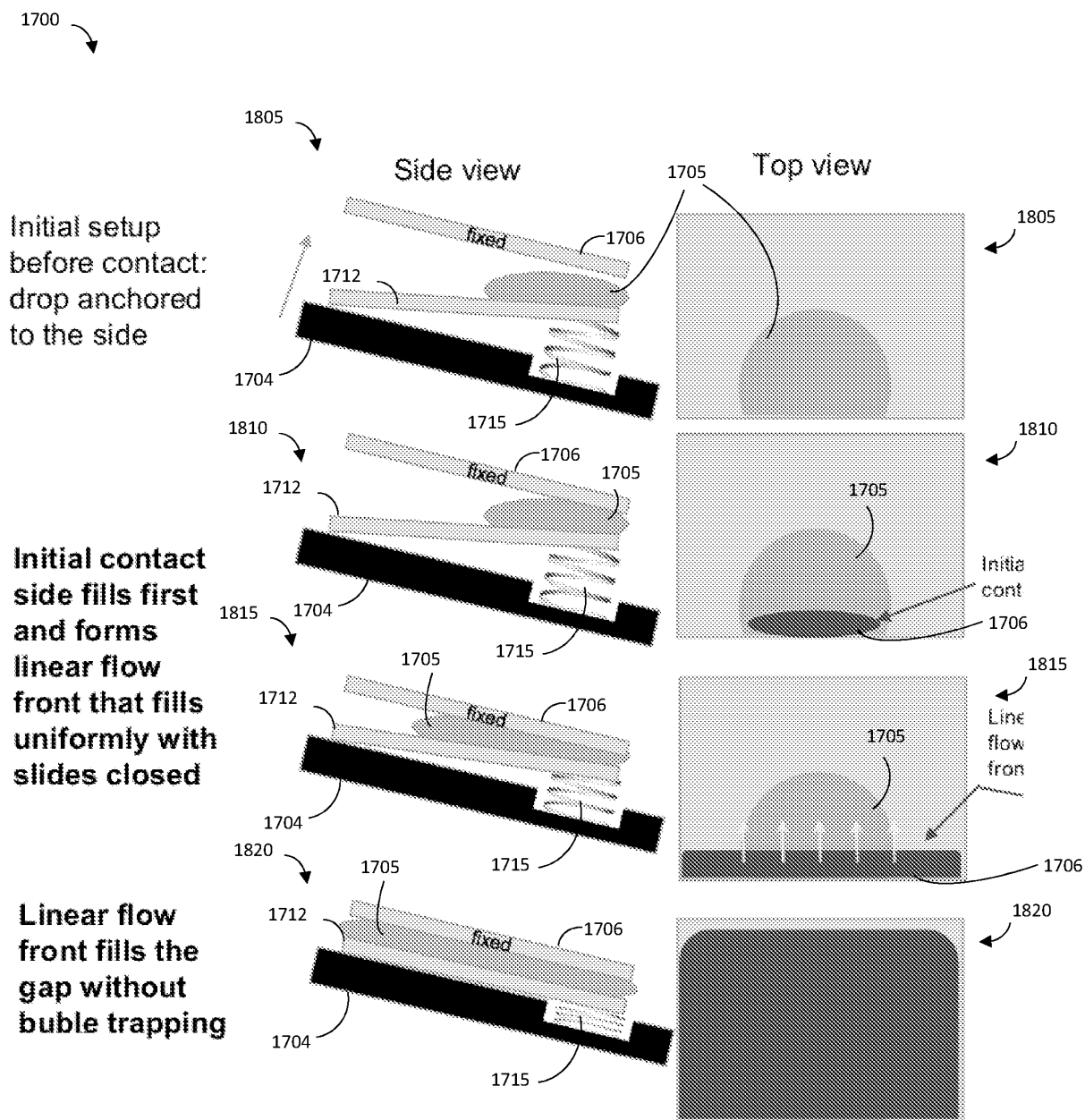


FIG. 18A

FIG. 18B

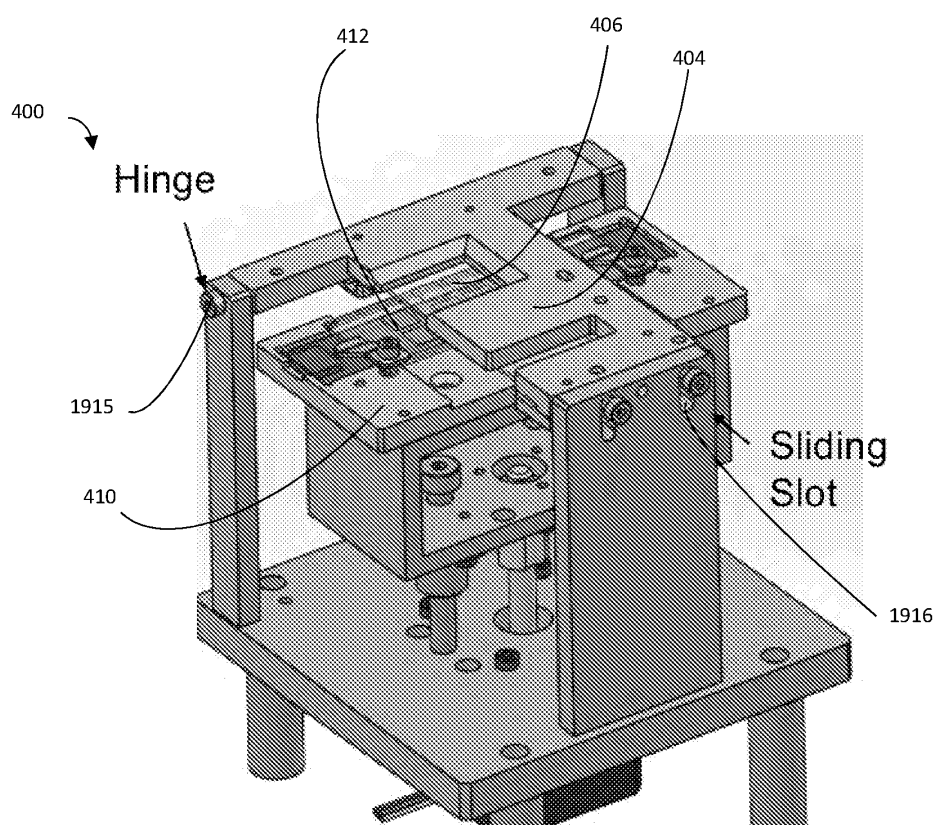


FIG. 19A

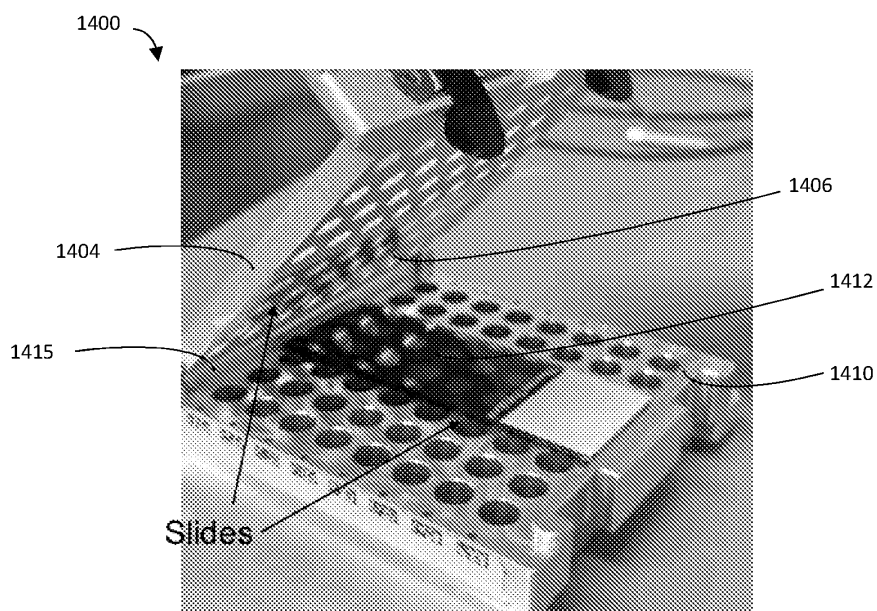


FIG. 19B

2000

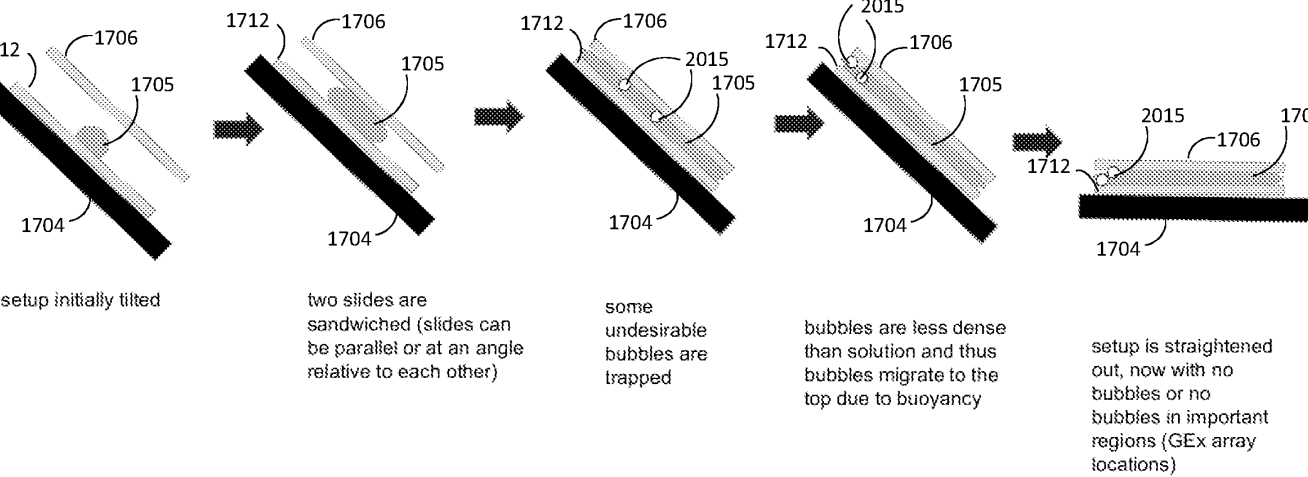


FIG. 20A

FIG. 20B

FIG. 20C

FIG. 20D

FIG. 20E

2100

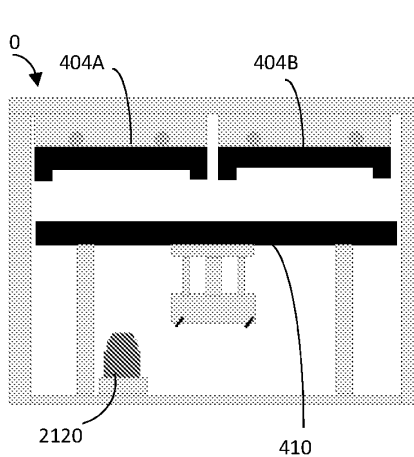


FIG. 21A

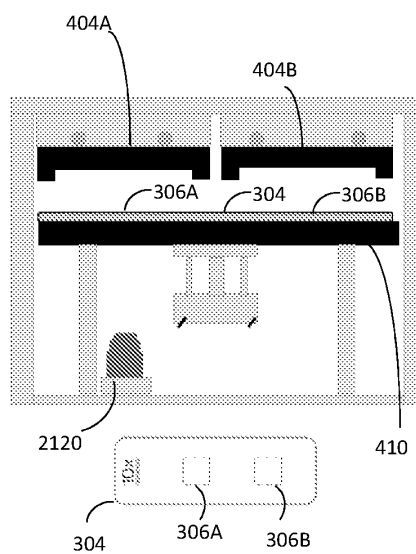


FIG. 21B

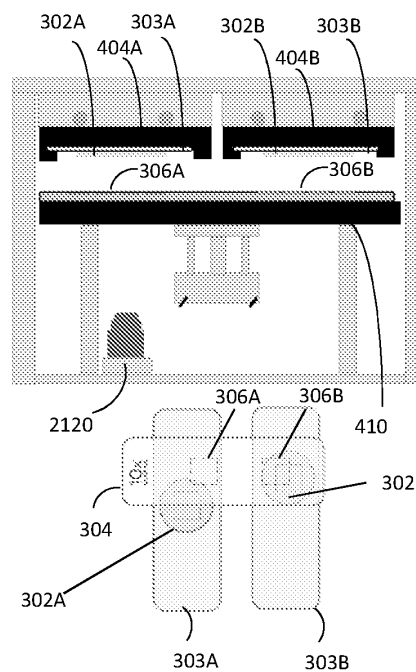


FIG. 21C

2200

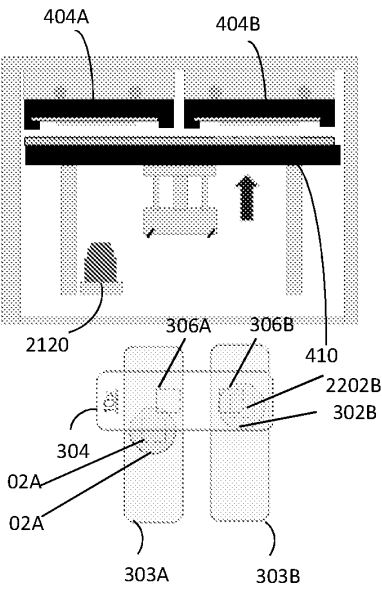


FIG. 22A

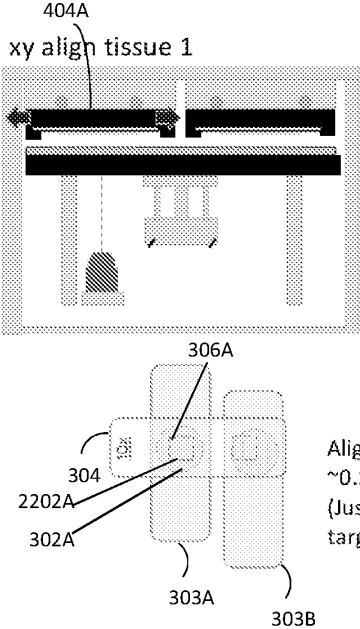


FIG. 22B

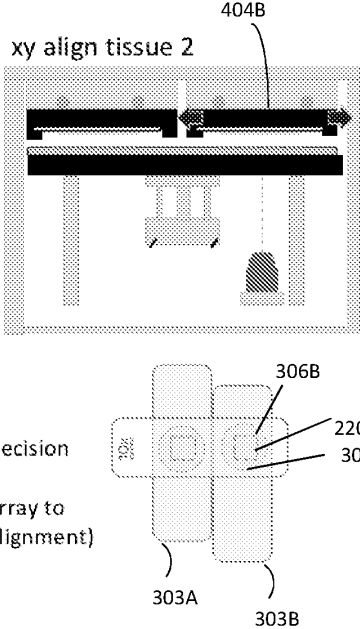


FIG. 22C

Alignment precision
~0.1-0.5 mm
(Just rough array to
target area alignment)

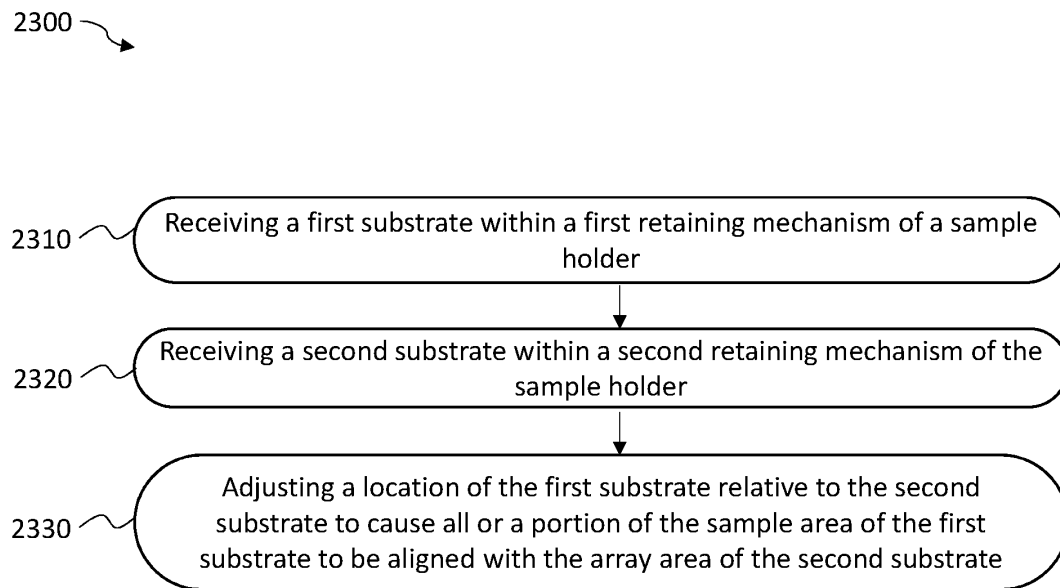


FIG. 23

2400

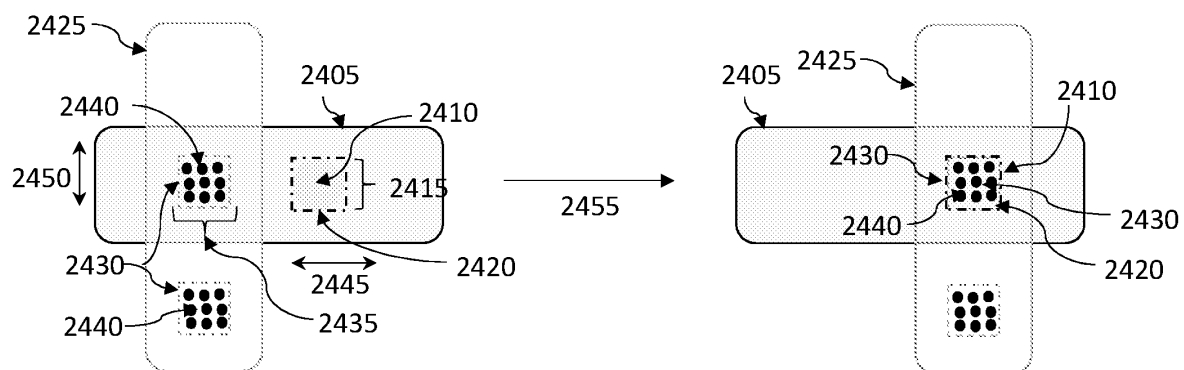


FIG. 24

2500

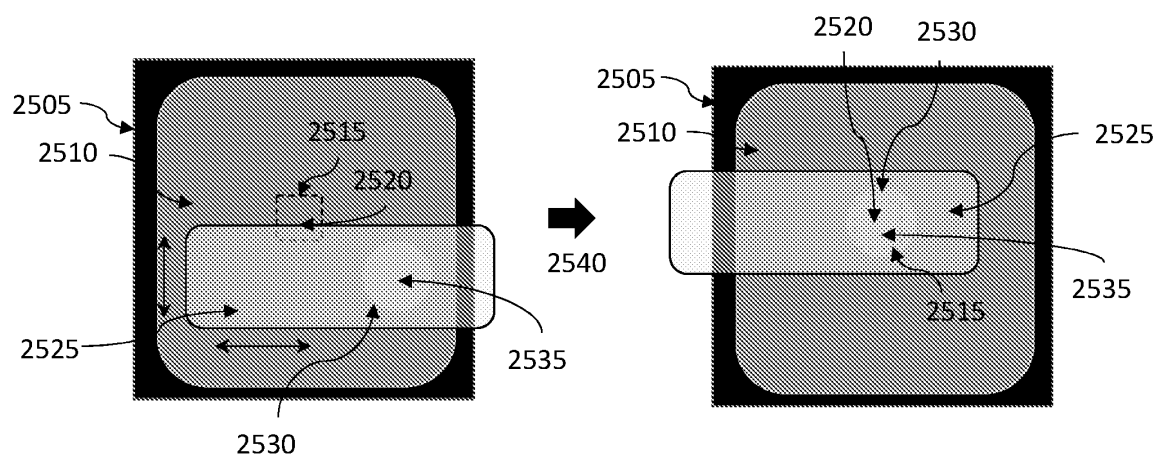


FIG. 25

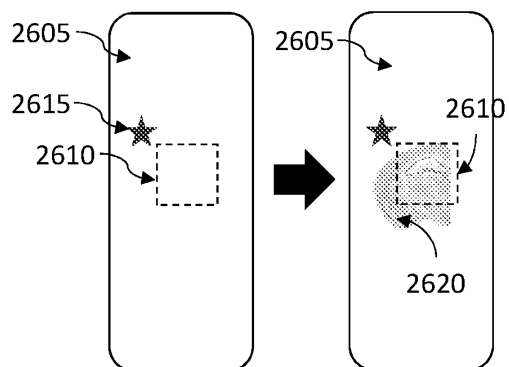


FIG. 26A

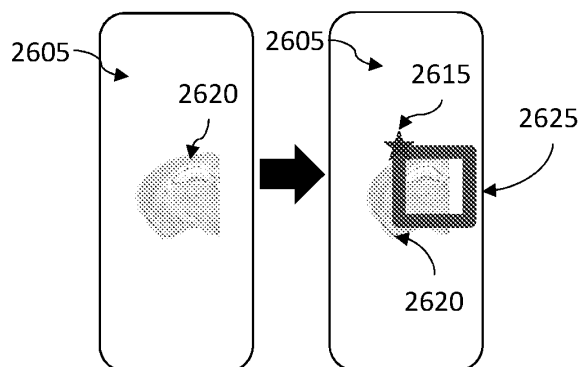


FIG. 26B

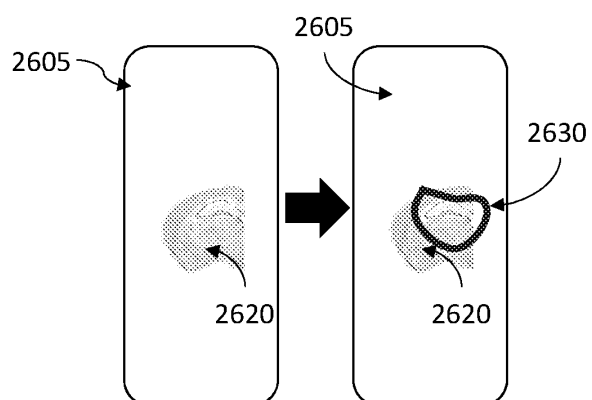


FIG. 26C

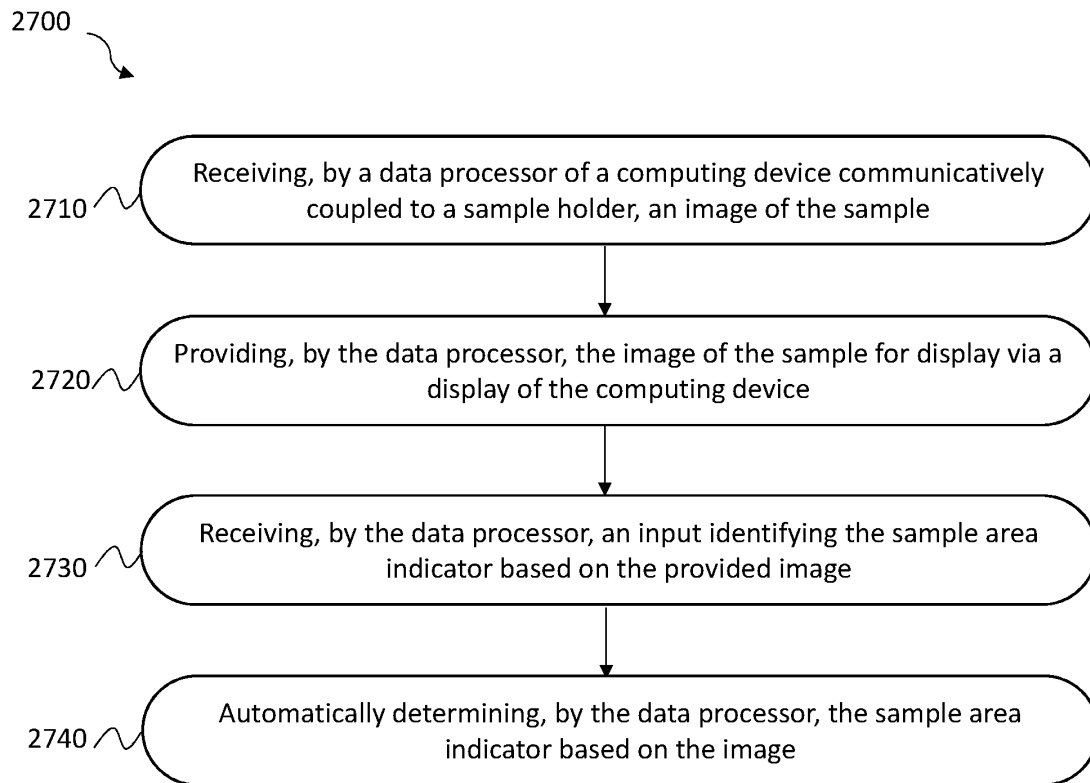


FIG. 27

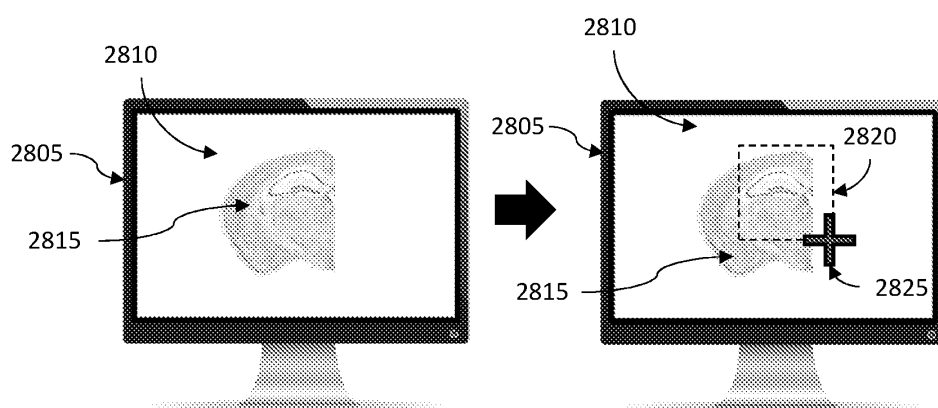


FIG. 28A

FIG. 28B

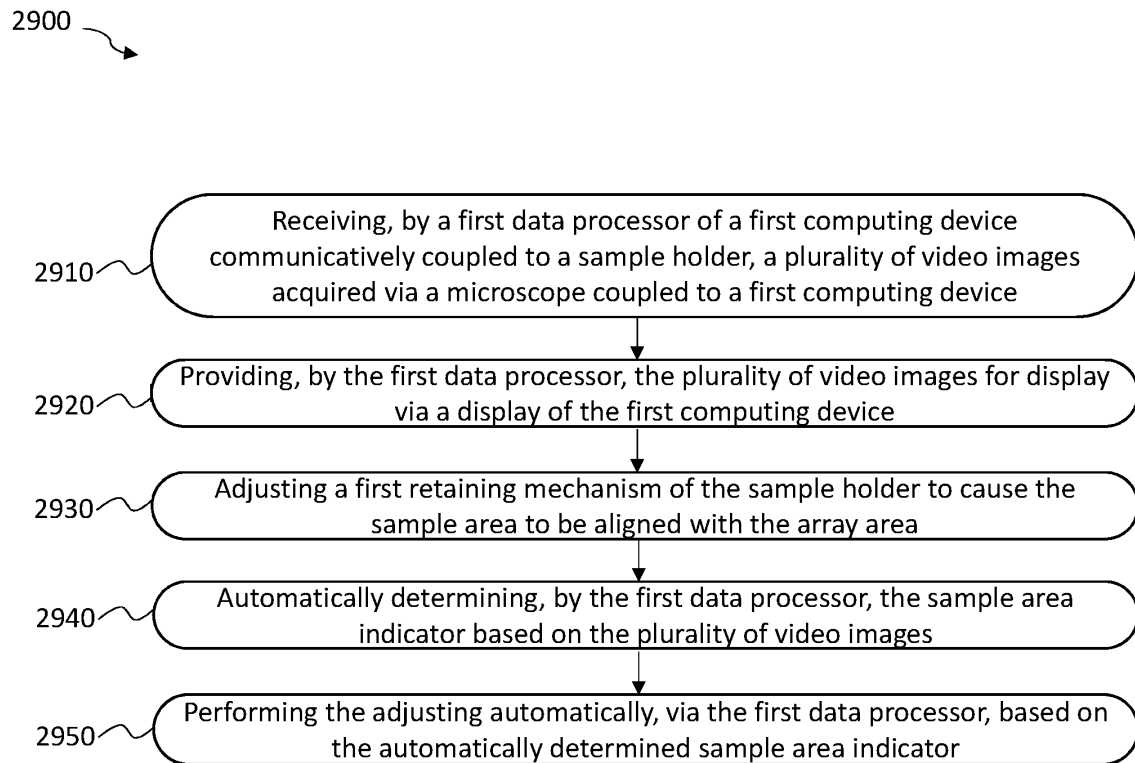


FIG. 29

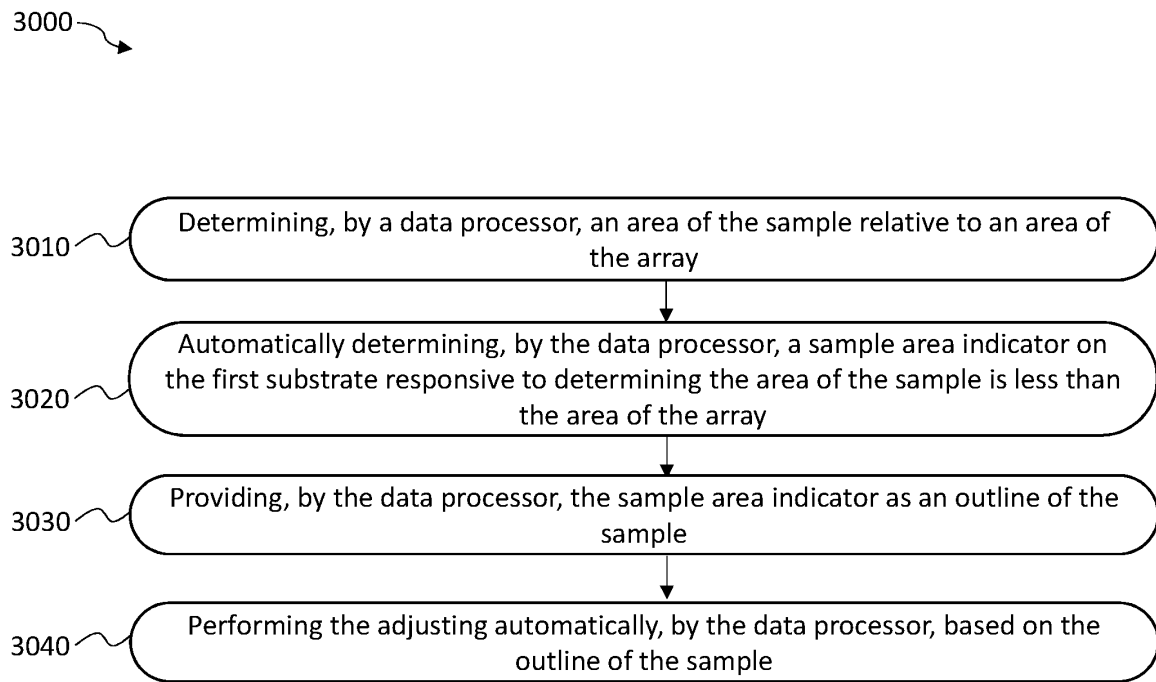


FIG. 30

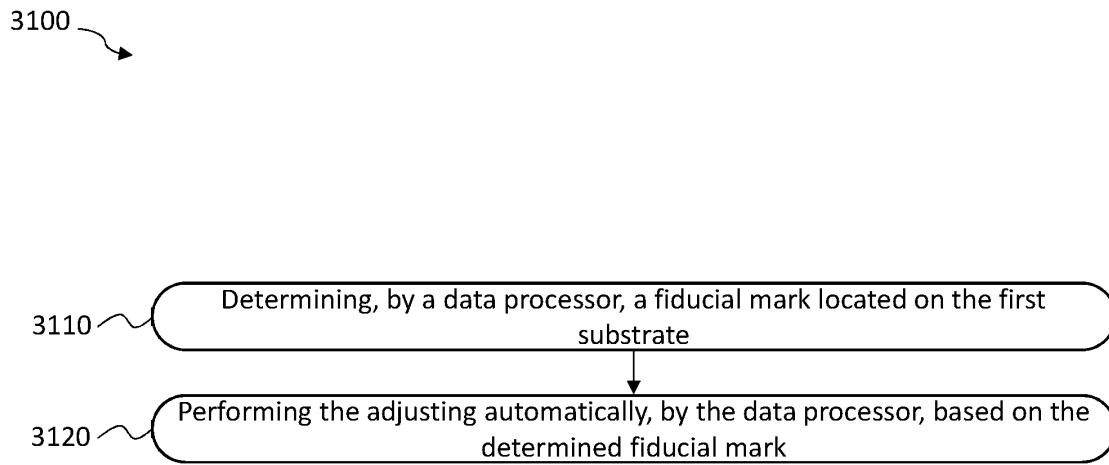


FIG. 31

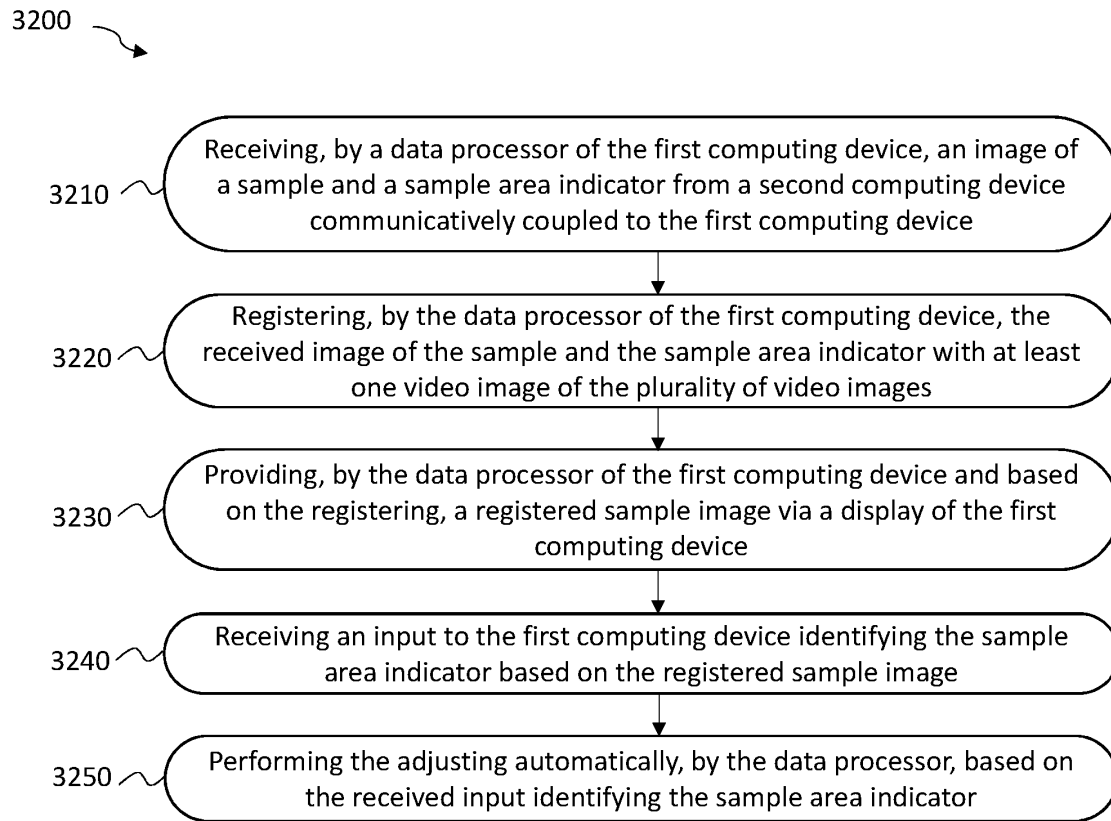


FIG. 32

3300

Permeabilization during Sandwich

Lower GEx slide

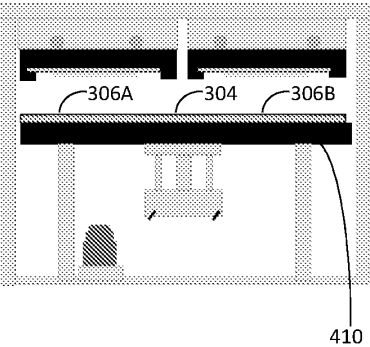


FIG. 33A

Perm solution is dispensed manually by user

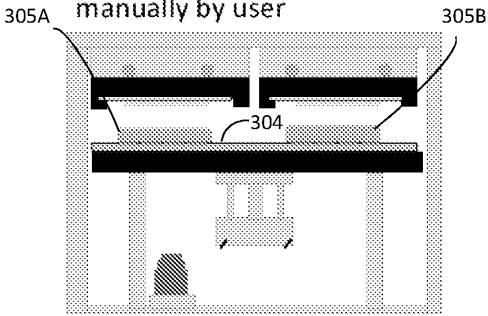


FIG. 33B

Sandwich is formed

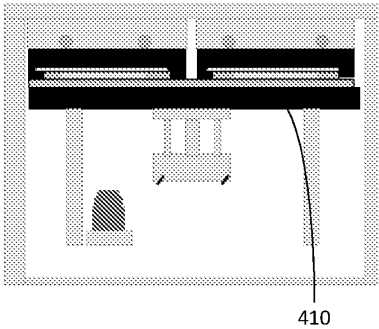
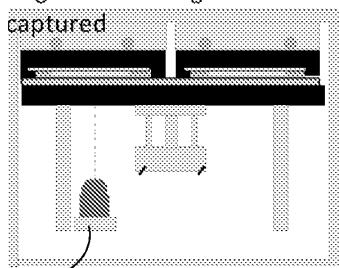


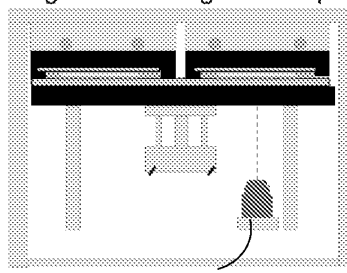
FIG. 33C

3400

Registration image capture (during the 1 min permeabilization)**Registration image #1 is captured**

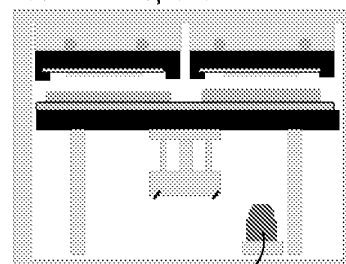
2120
Alignment precision <10
microns
Need to record alignment of
fiducials on GEx slide to tissue

FIG. 34A

Registration image #2 is captured

2120
reg image2
3422

FIG. 34B

Sandwich opened

2120
GEx slide needs to be quickly removed
for wash or wash solution loaded into
the instrument for washing

FIG. 34C

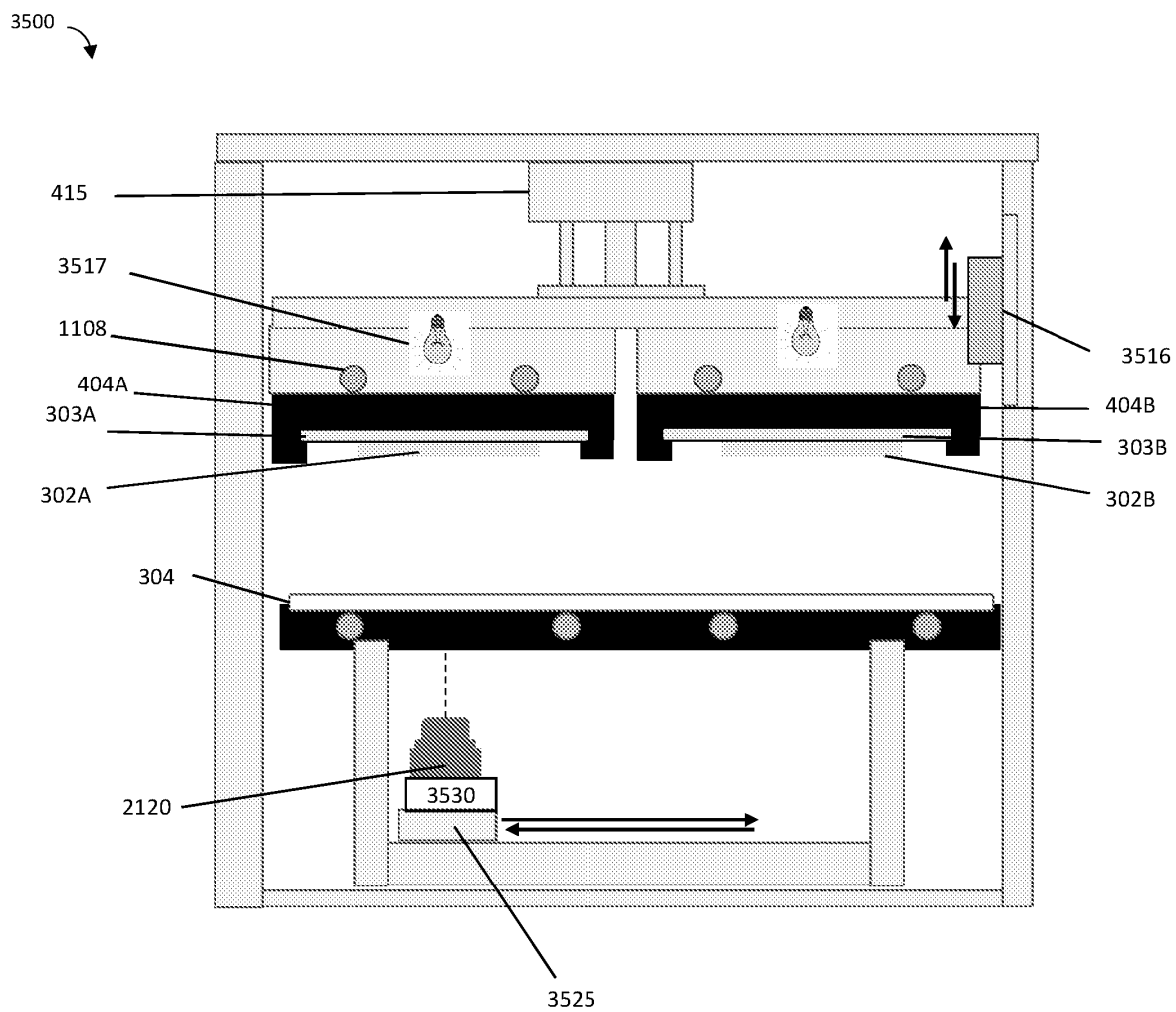


FIG. 35

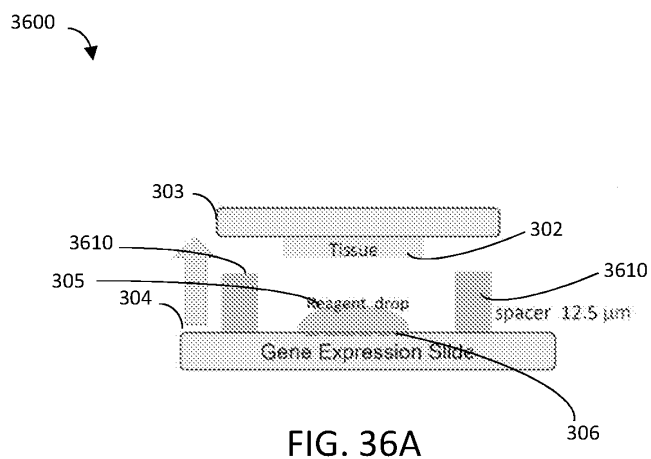


FIG. 36A

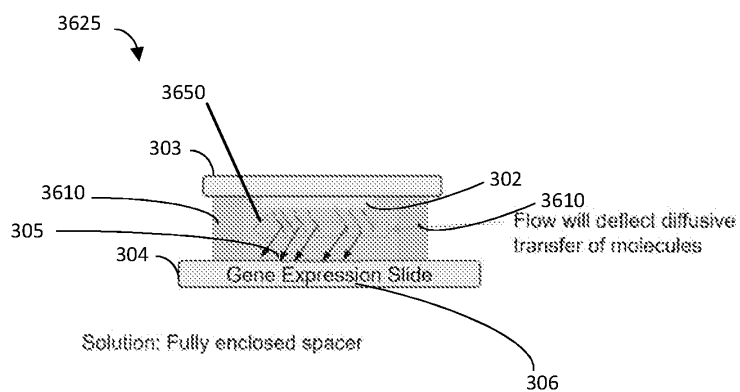


FIG. 36B

Solution: Fully enclosed spacer

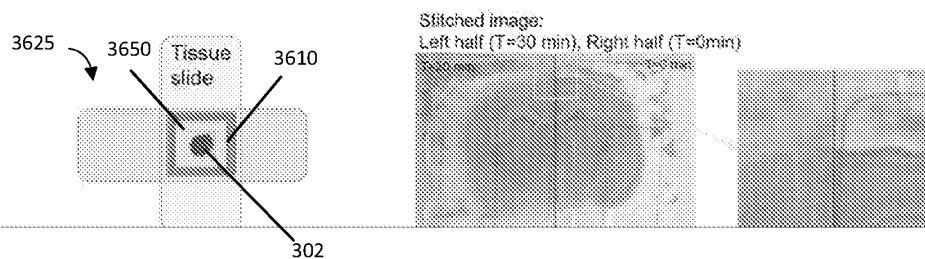


FIG. 36C

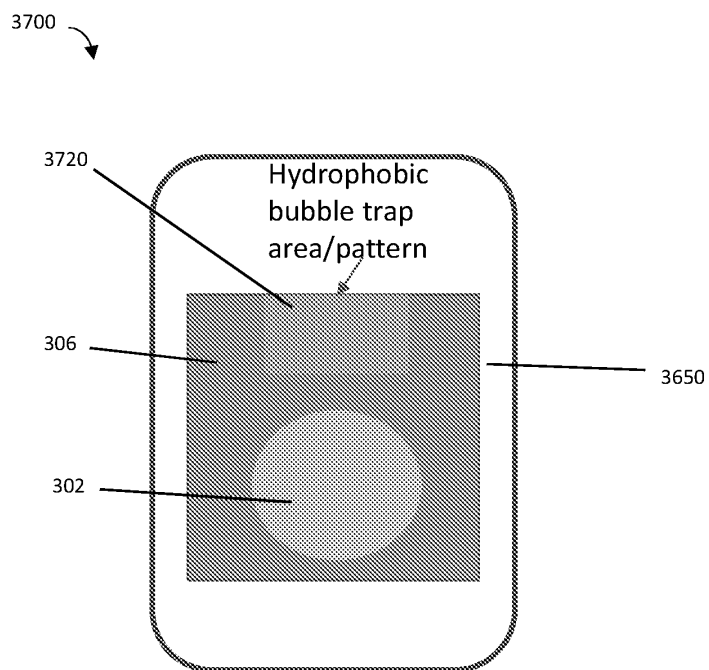


FIG. 37

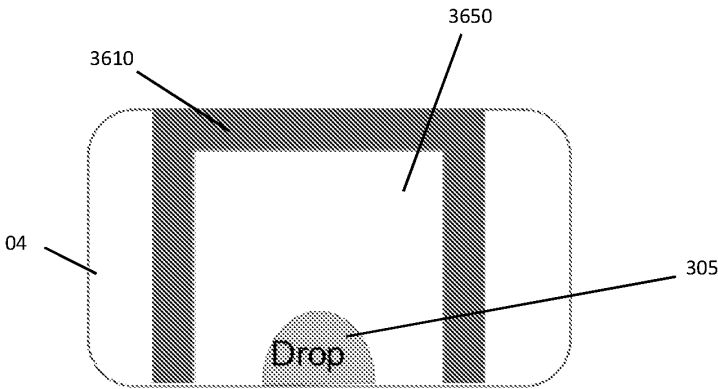


FIG. 38A

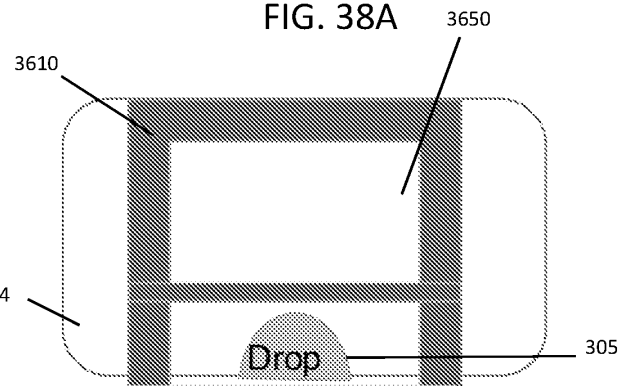


FIG. 38B

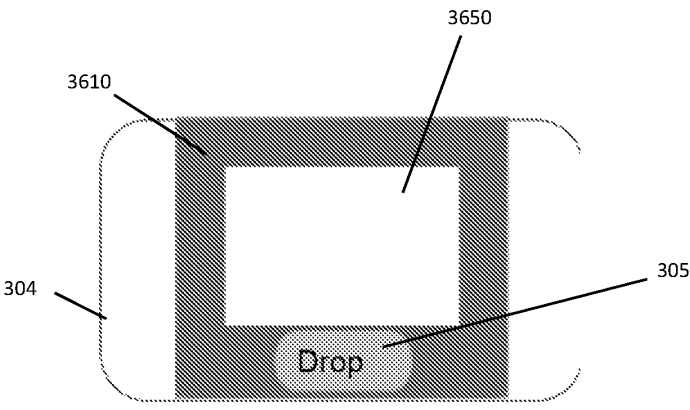


FIG. 38C

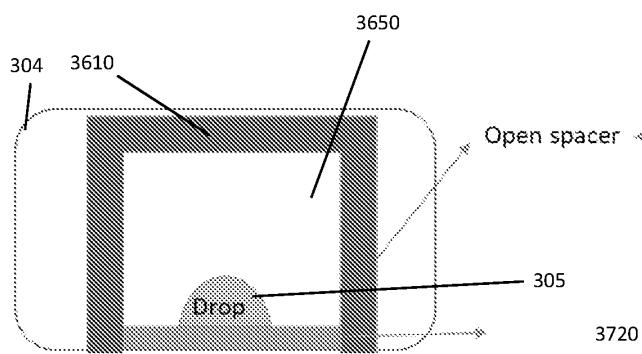


FIG. 39A

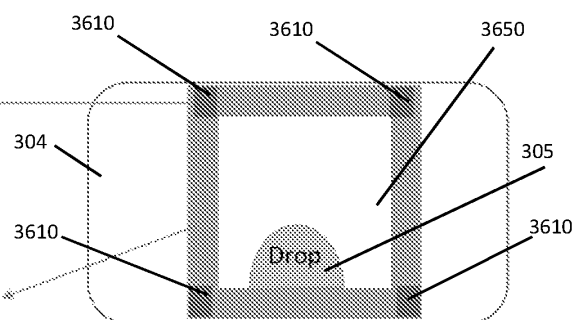


FIG. 39B

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

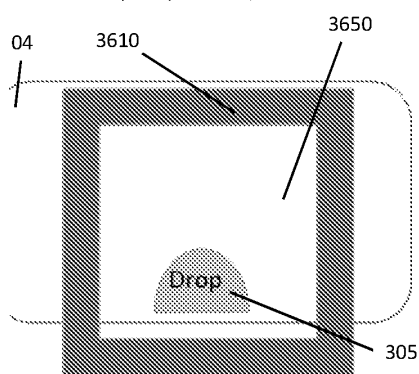


FIG. 39C

+

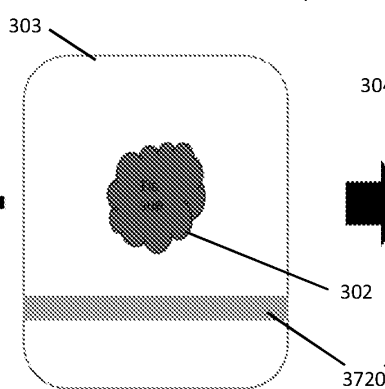


FIG. 39D

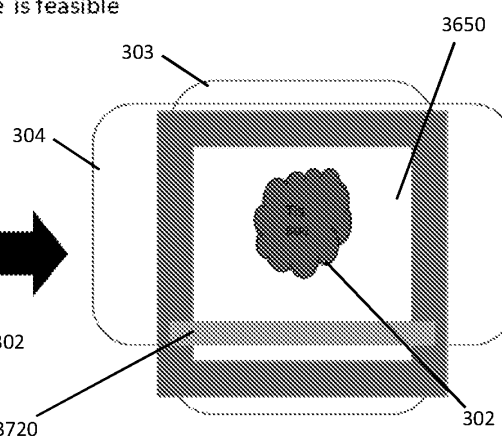
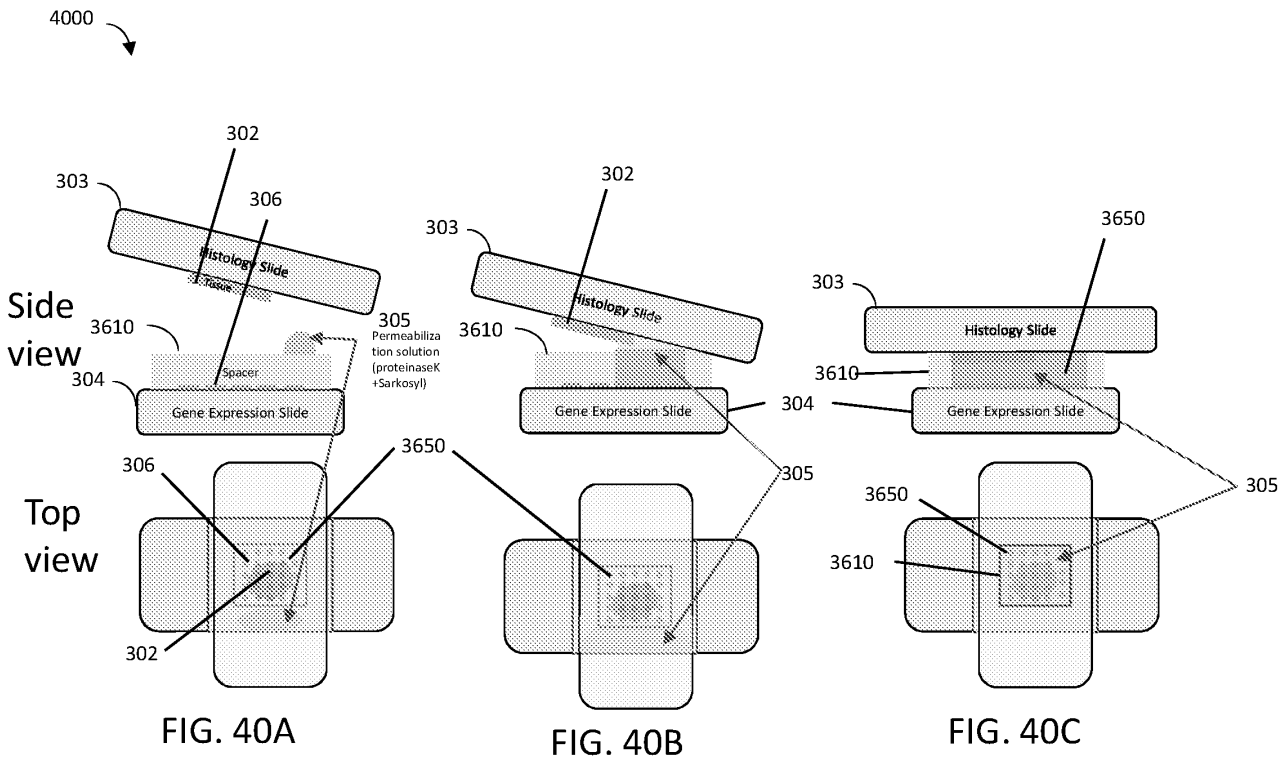
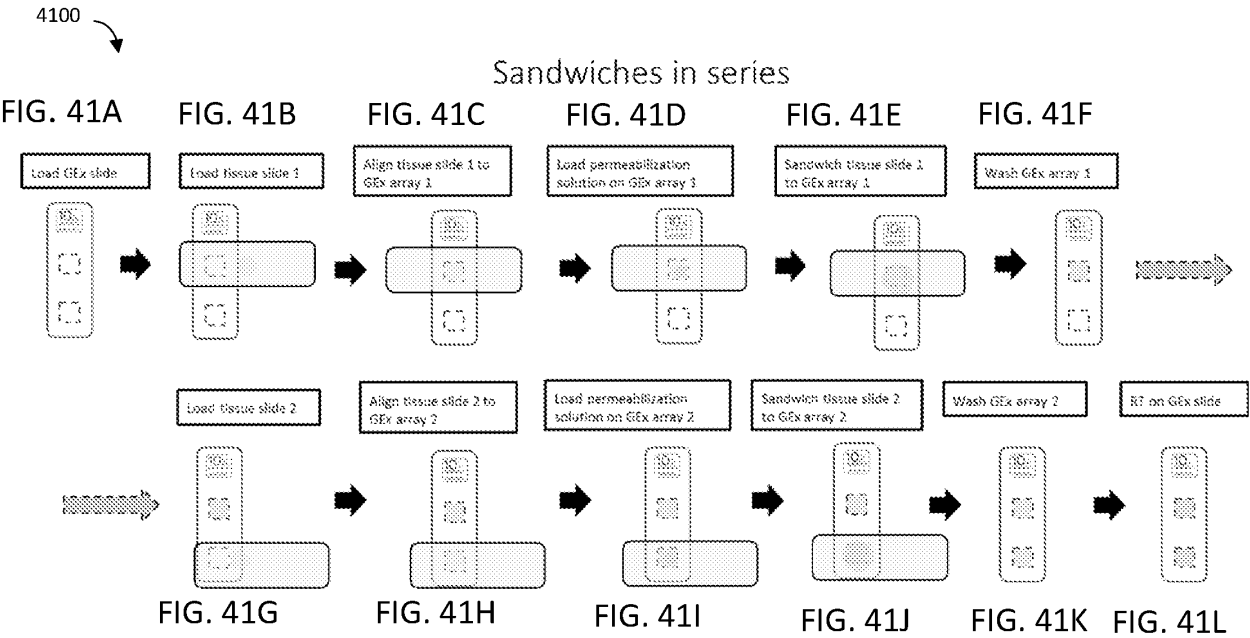


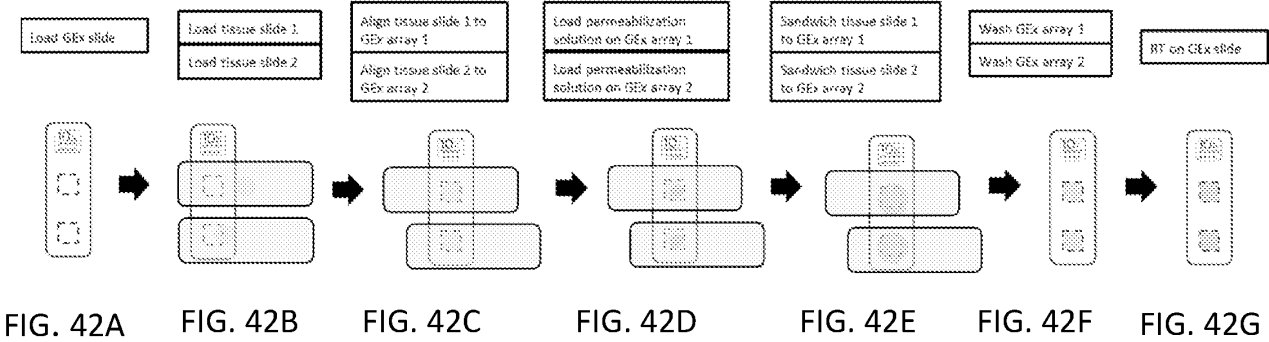
FIG. 39E





4200

Parallel Sandwiches



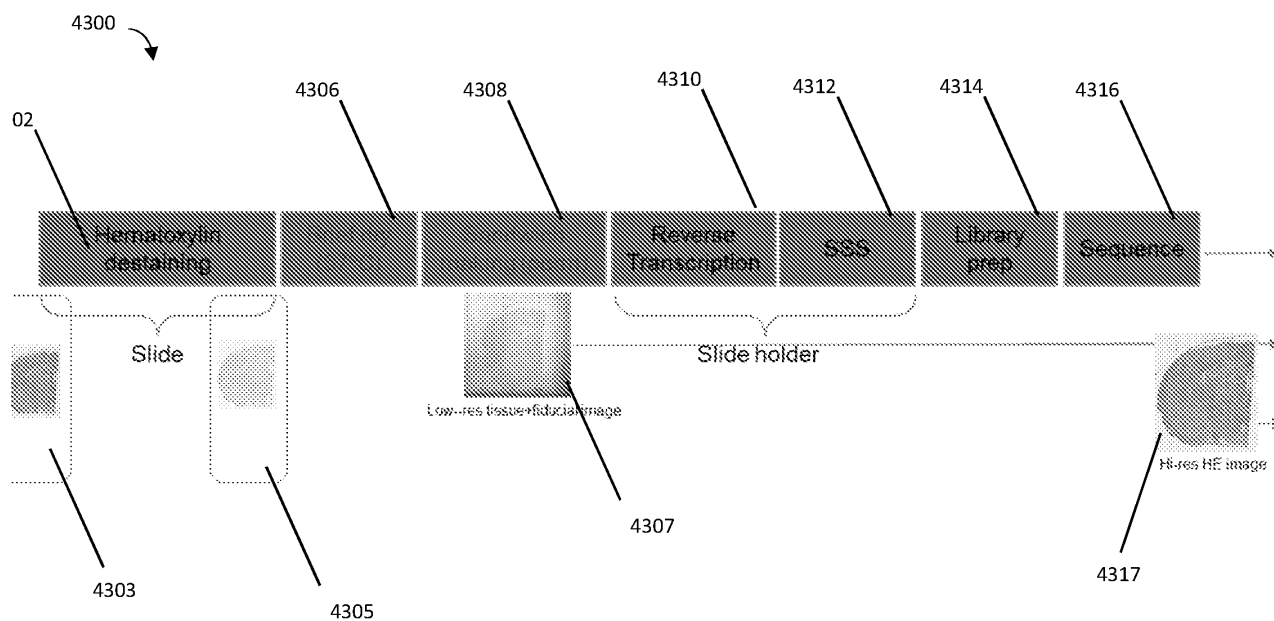


FIG. 43

4400

Analysis ID	Sample	Description	Permeabilization time	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 control	5 minutes	5194	17660
1046322				4592	13083
1046514		Permeabilization in sandwich assembly	1 minute	4072	14017
1046515				3758	13731

FIG. 44

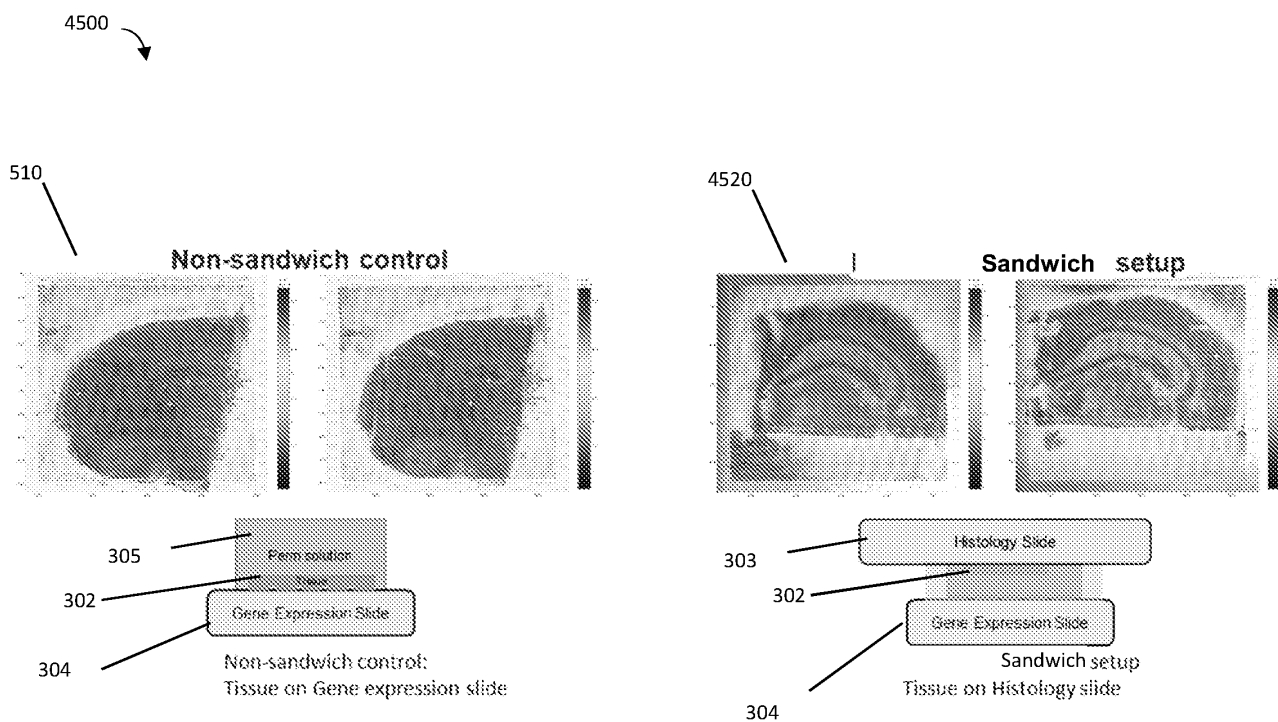


FIG. 45

4600

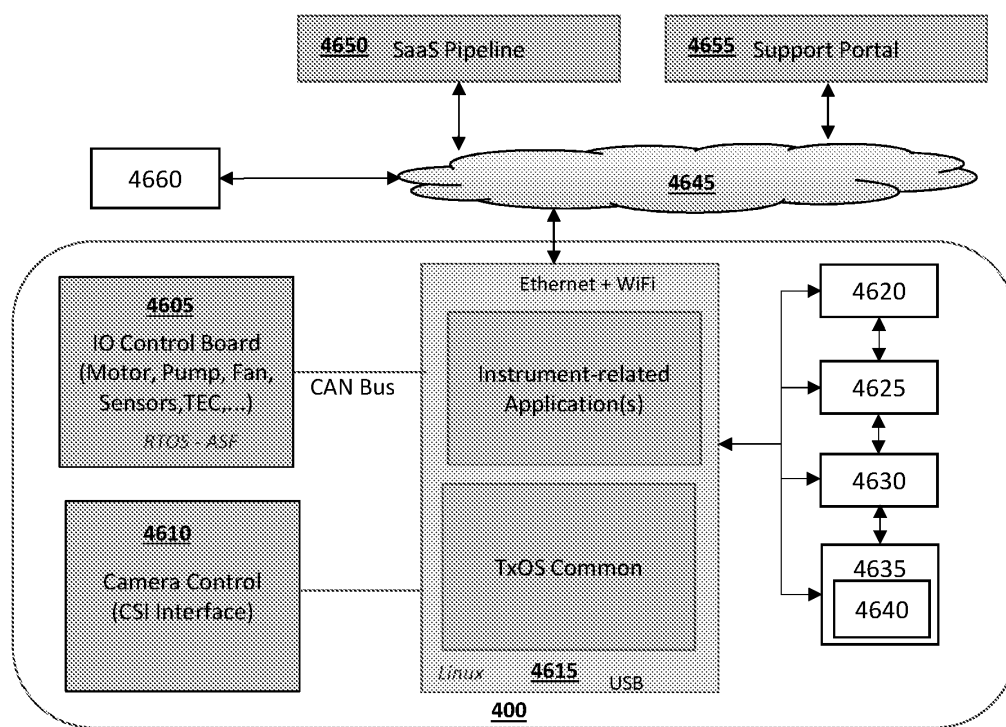


FIG. 46

4700

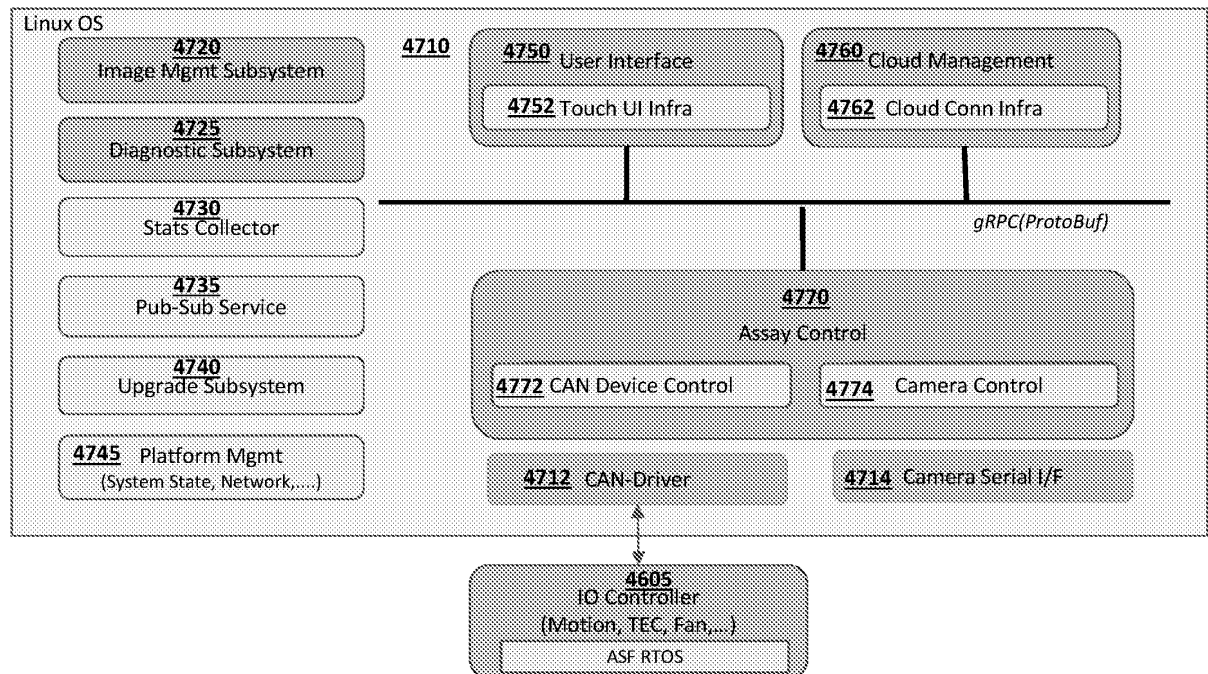


FIG. 47

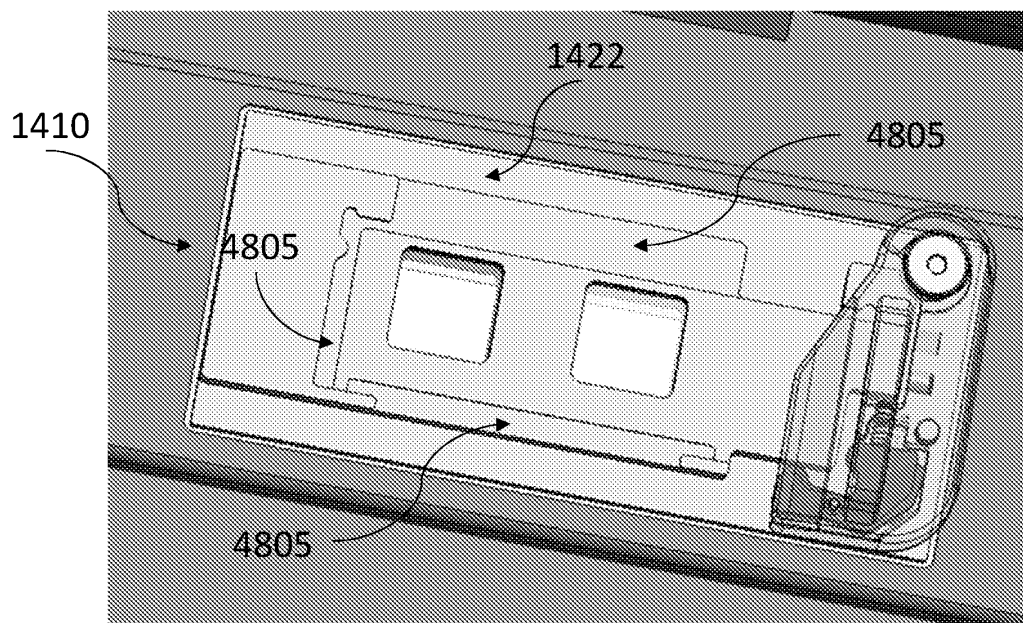


FIG. 48A

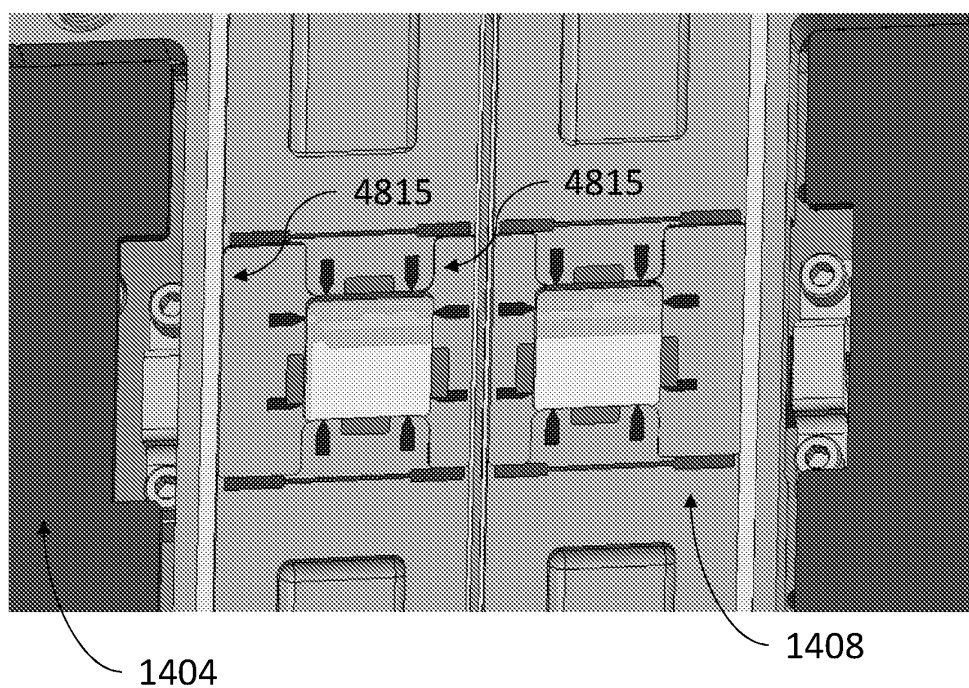


FIG. 48B

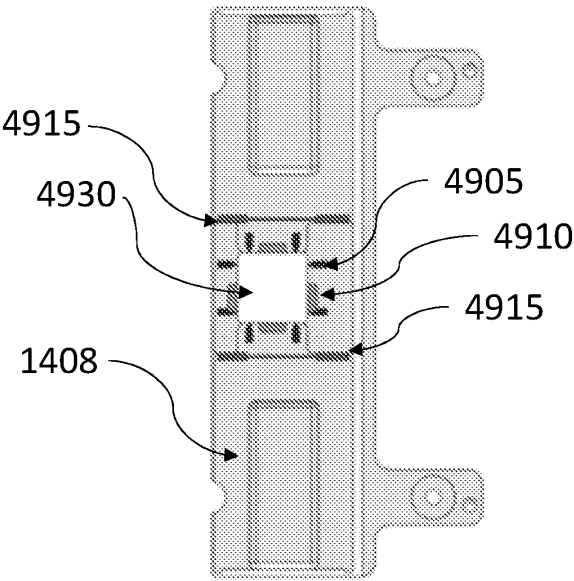


FIG. 49A

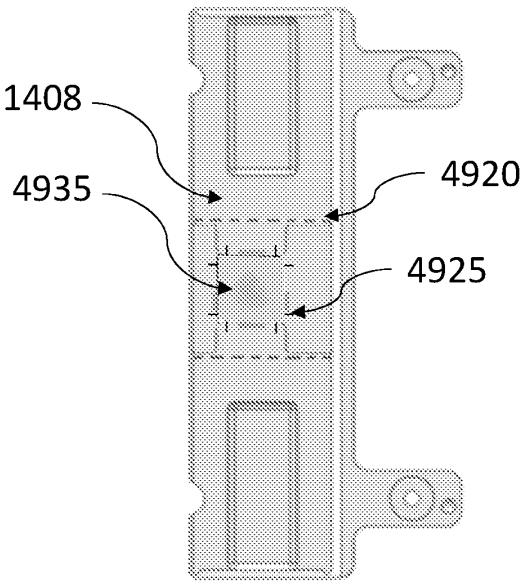


FIG. 49B

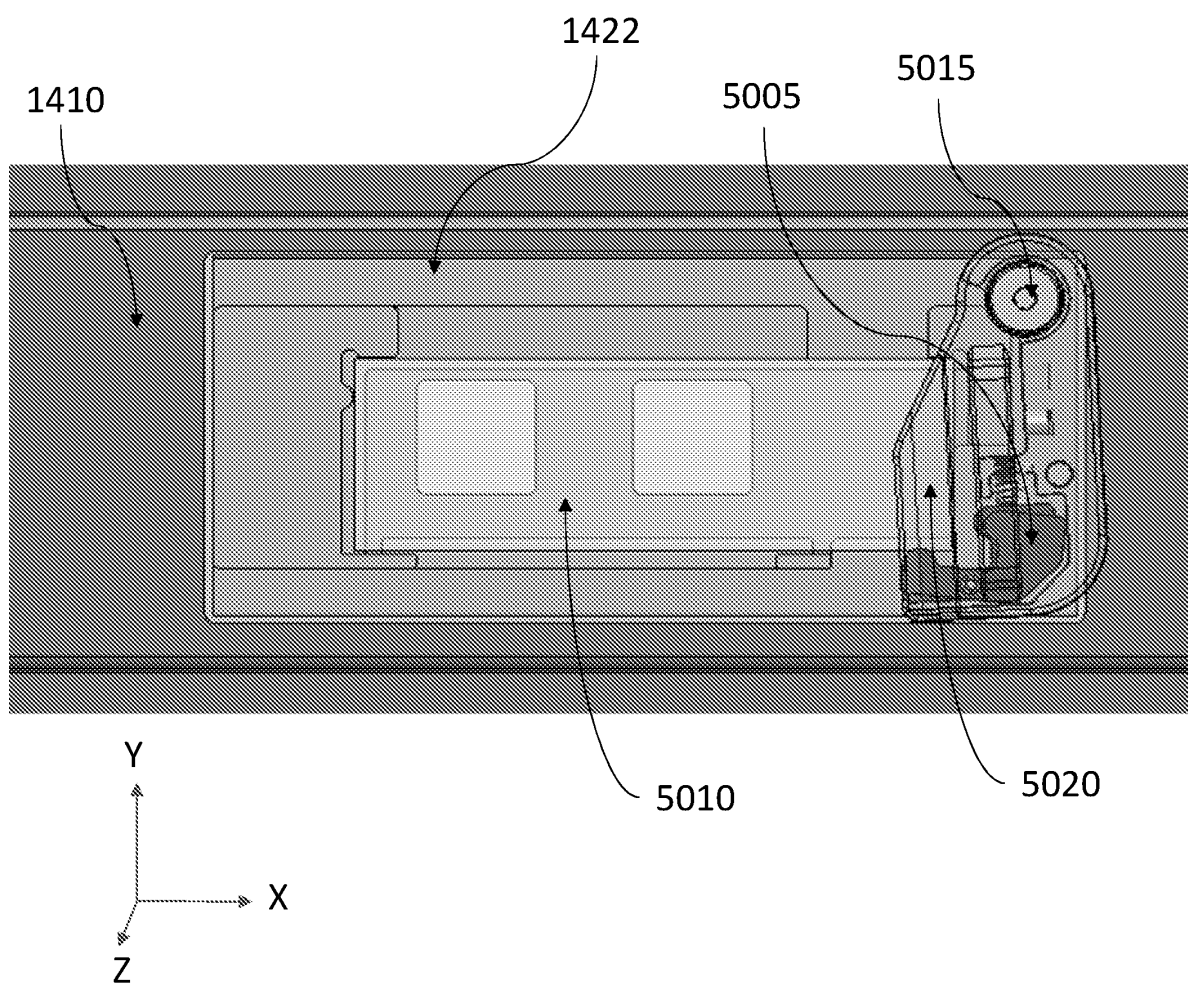


FIG. 50

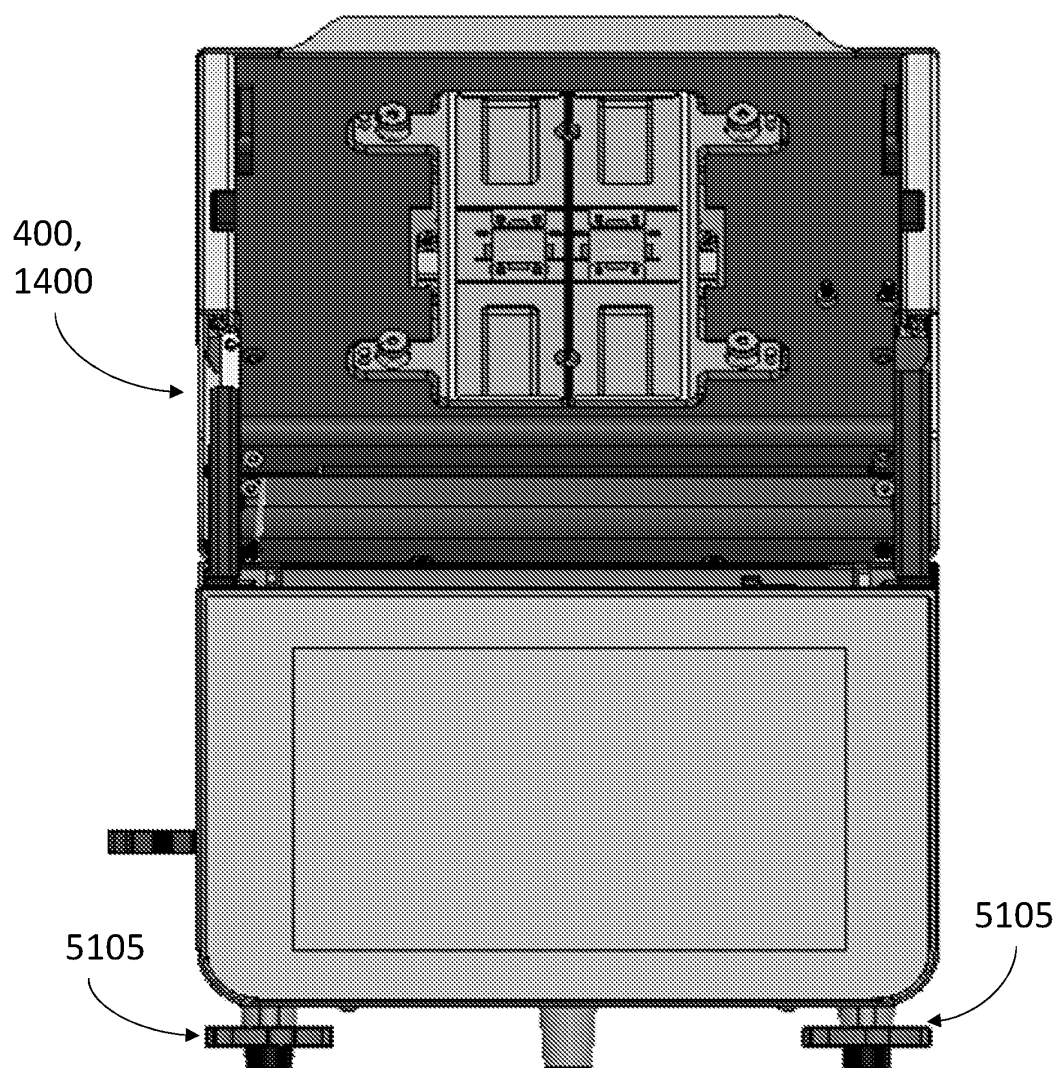


FIG. 51

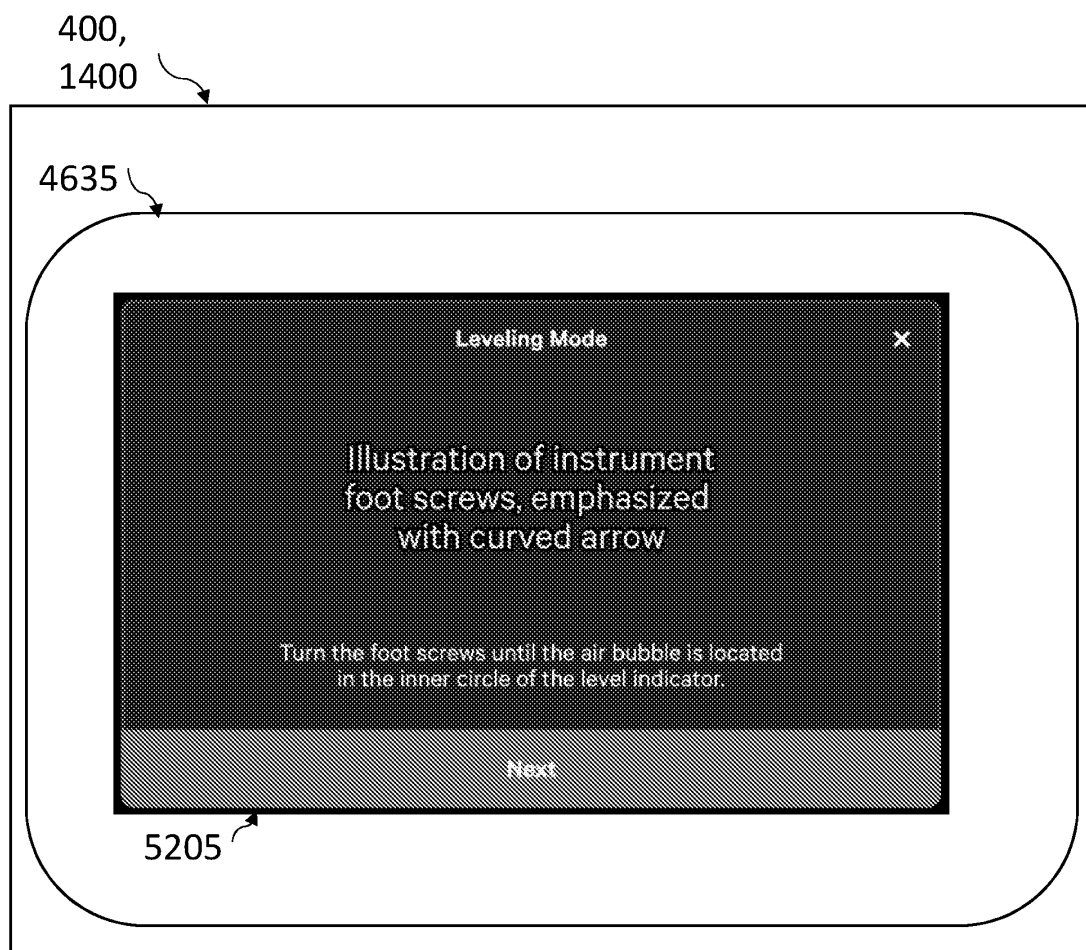
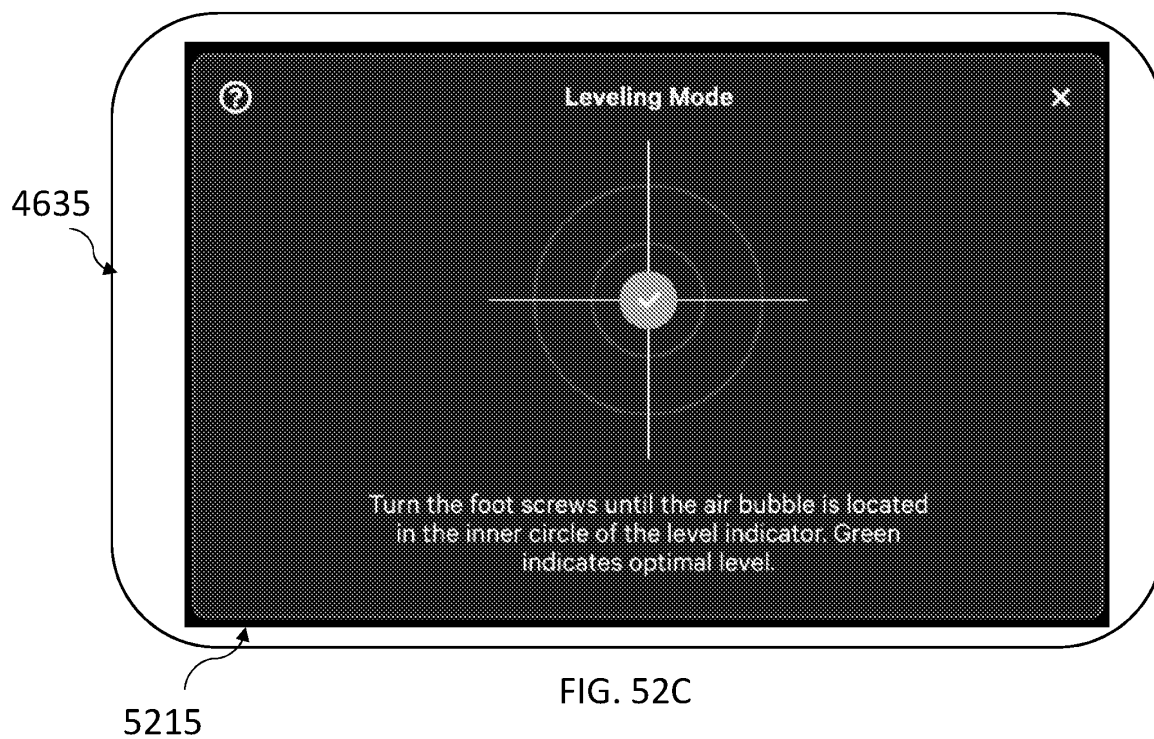
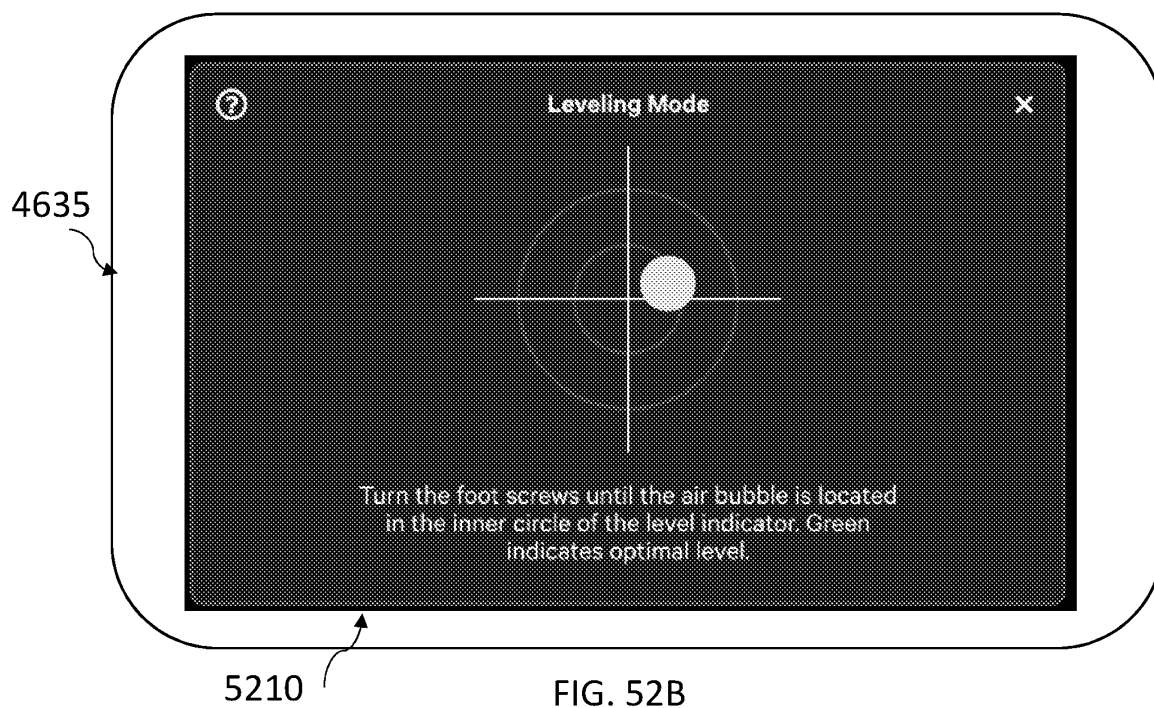


FIG. 52A



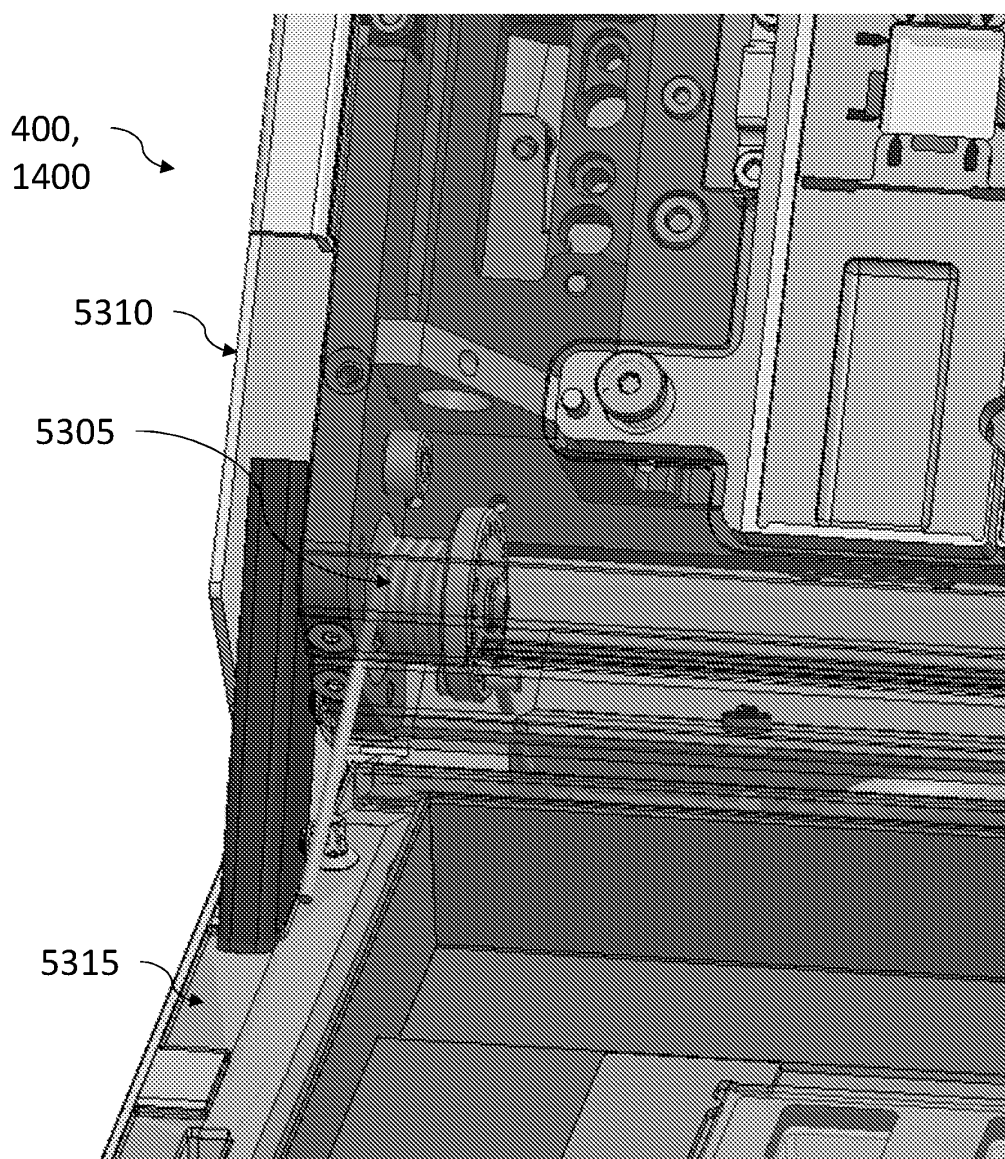


FIG. 53

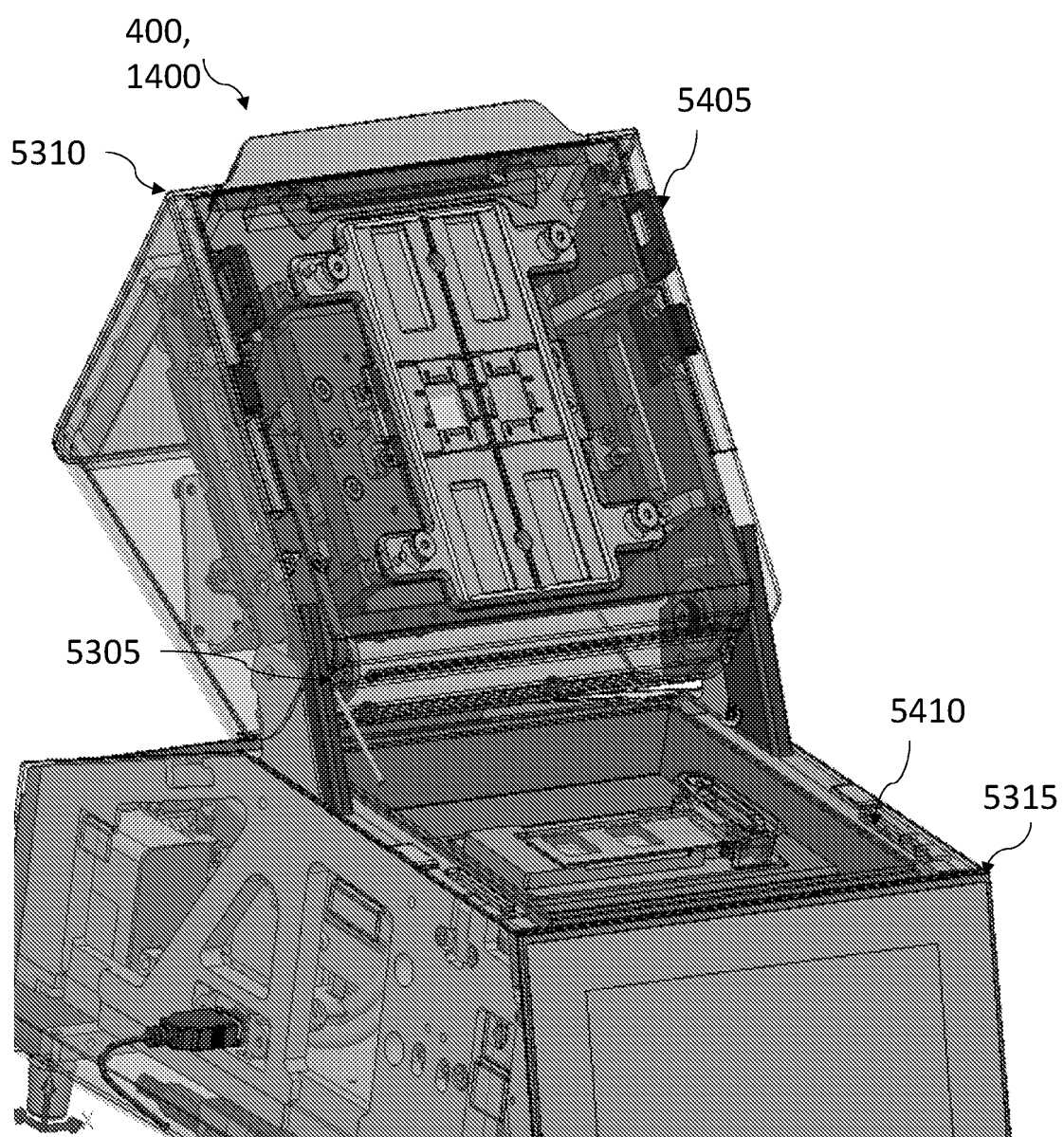


FIG. 54A

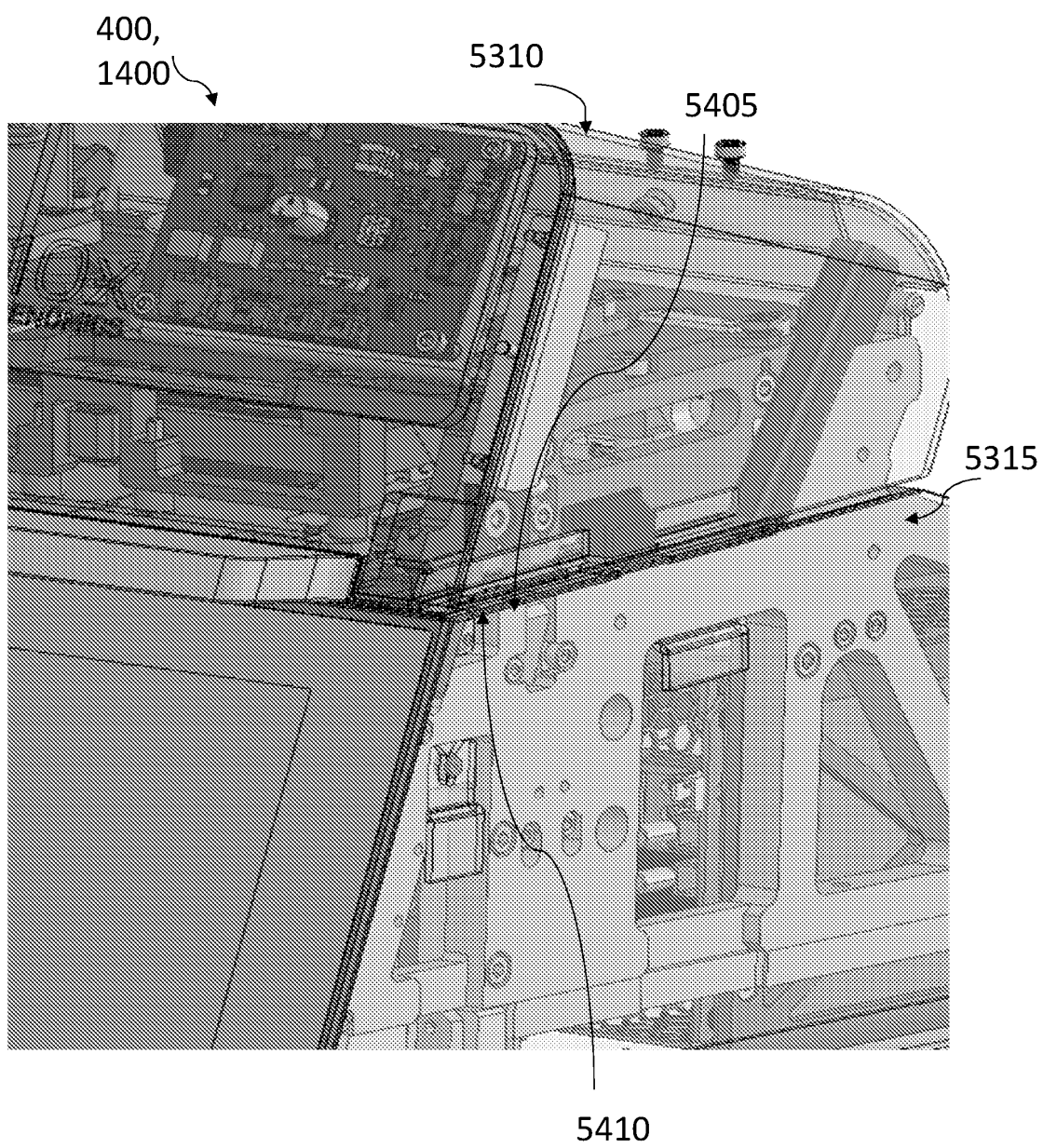


FIG. 54B

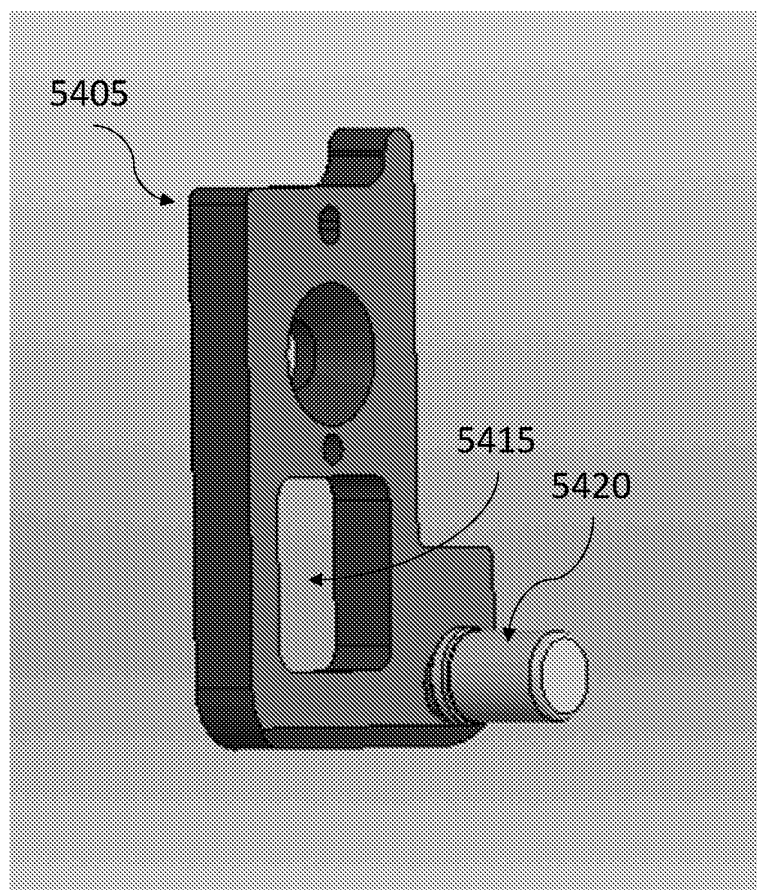


FIG. 54C

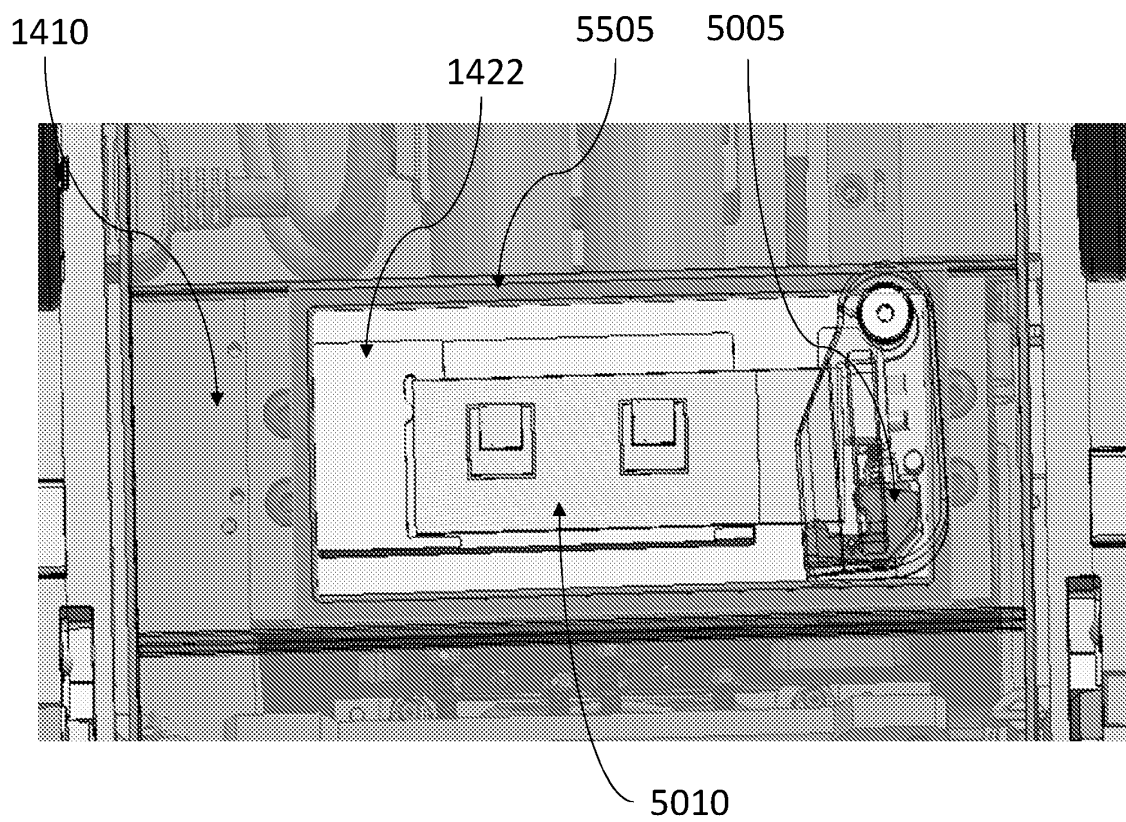


FIG. 55

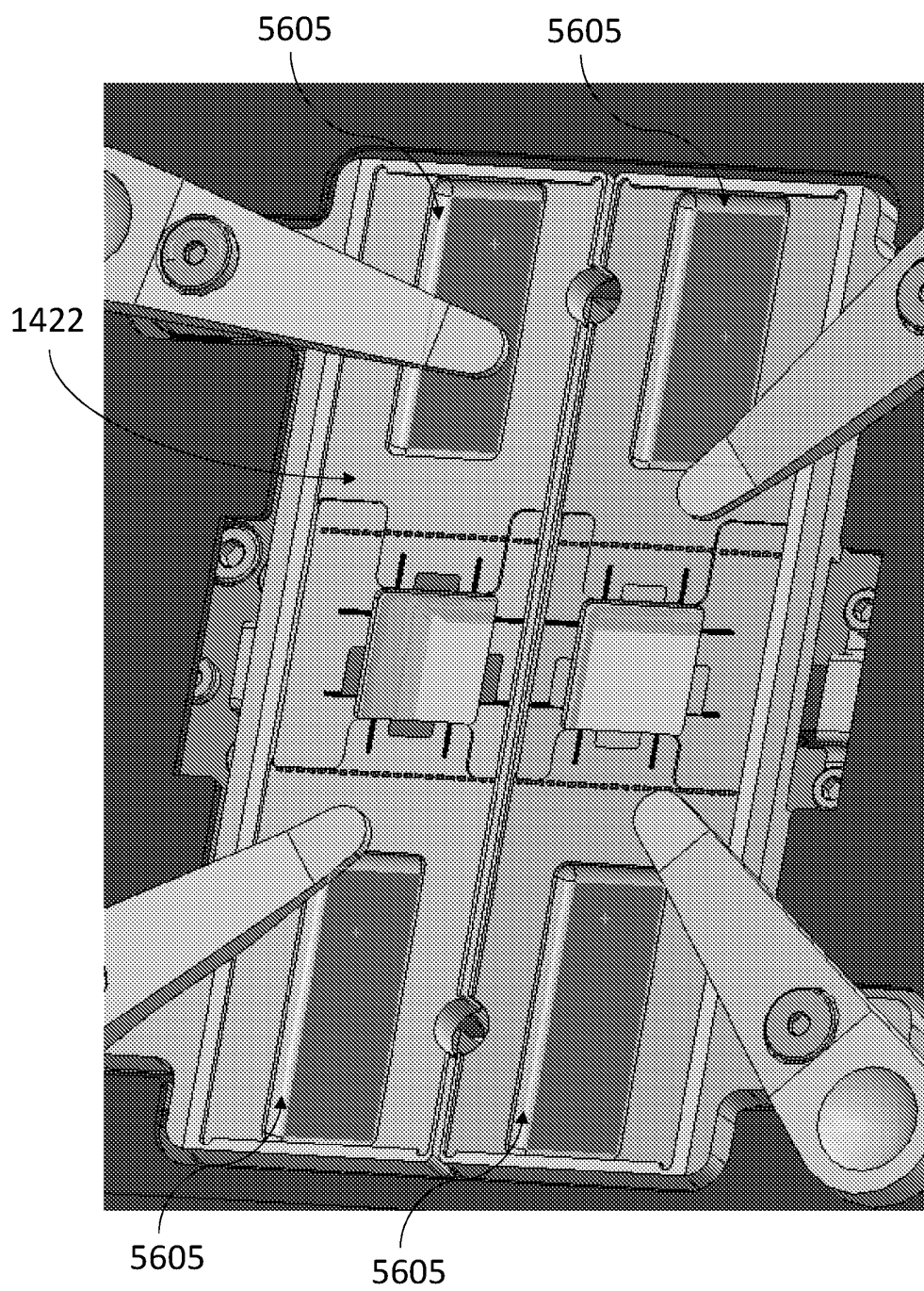


FIG. 56

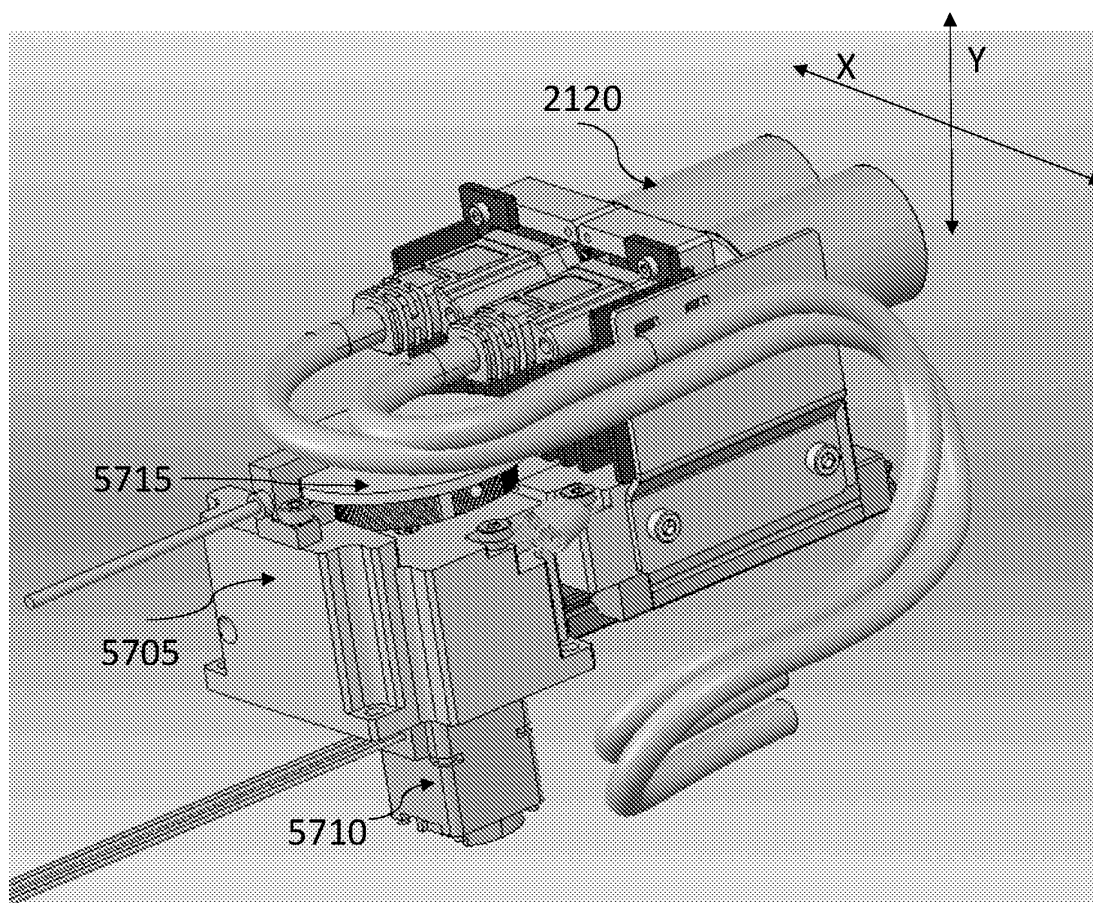


FIG. 57

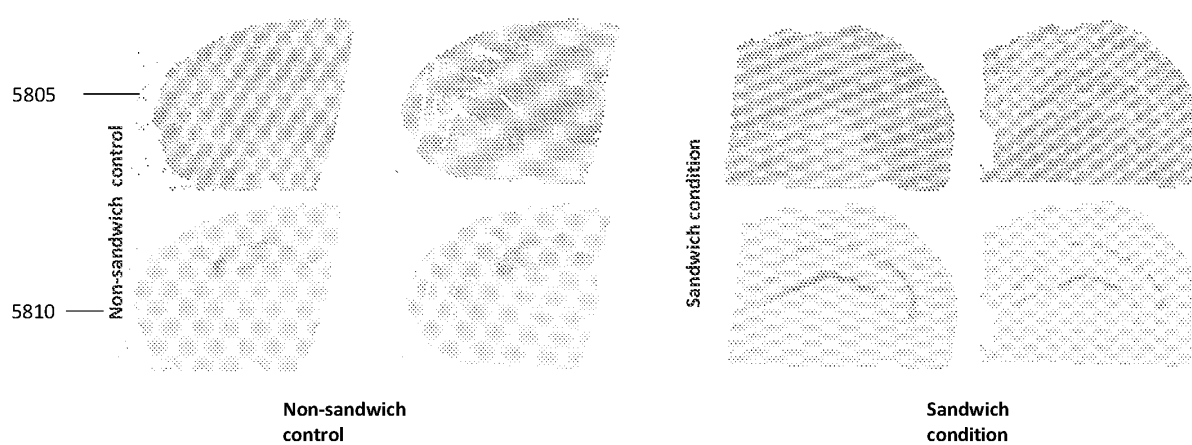


FIG. 58

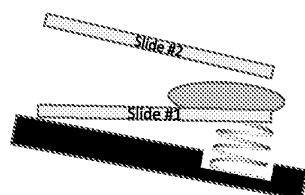
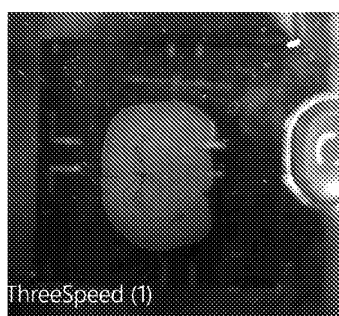


FIG. 59A

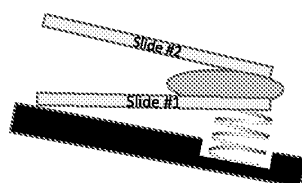
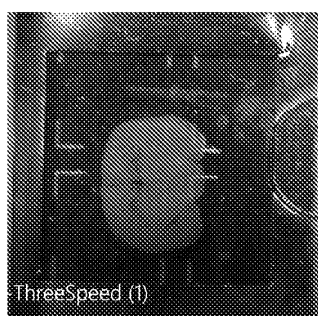


FIG. 59B

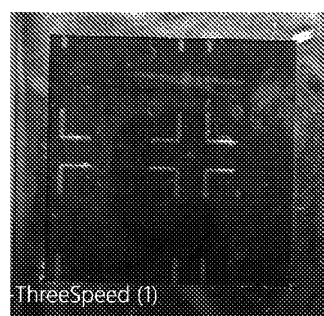


FIG. 59C

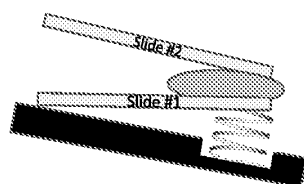
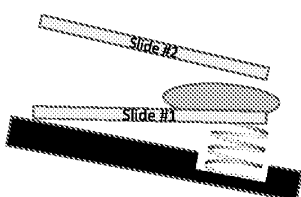
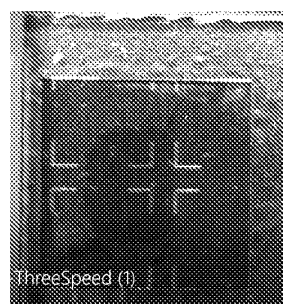
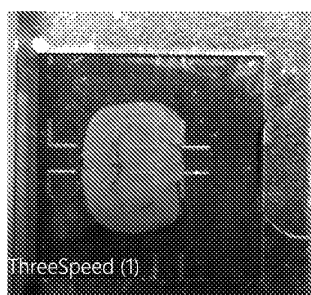
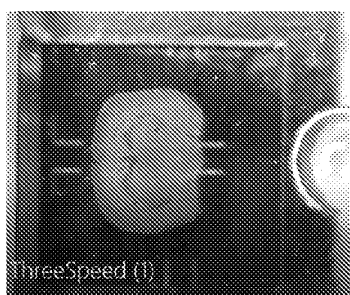


FIG. 60A

FIG. 60B

FIG. 60C

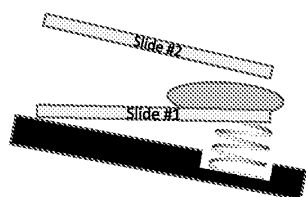
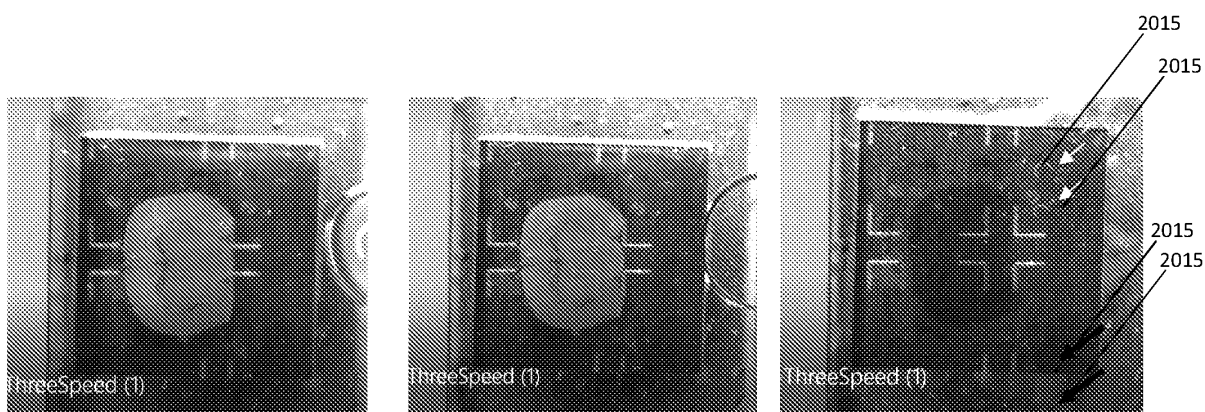


FIG. 61A

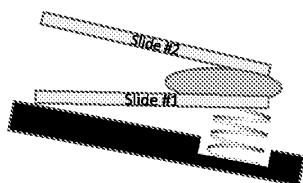


FIG. 61B

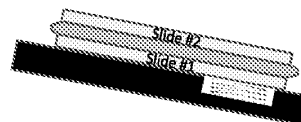


FIG. 61C

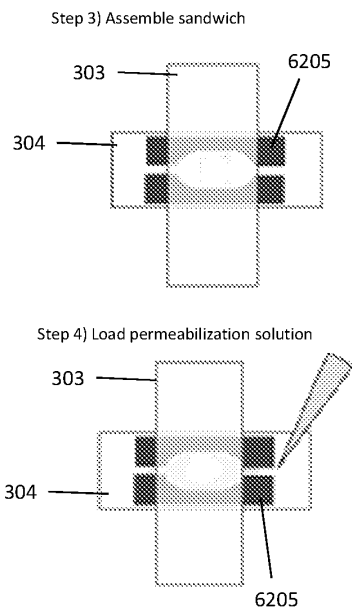
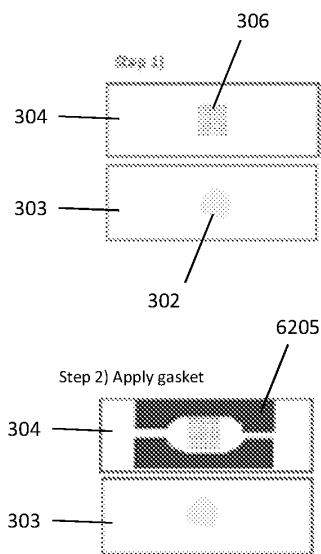


FIG. 62A

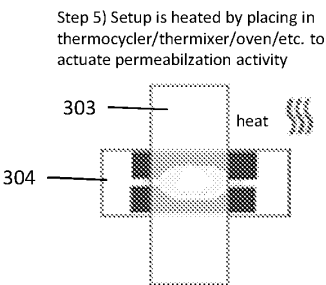
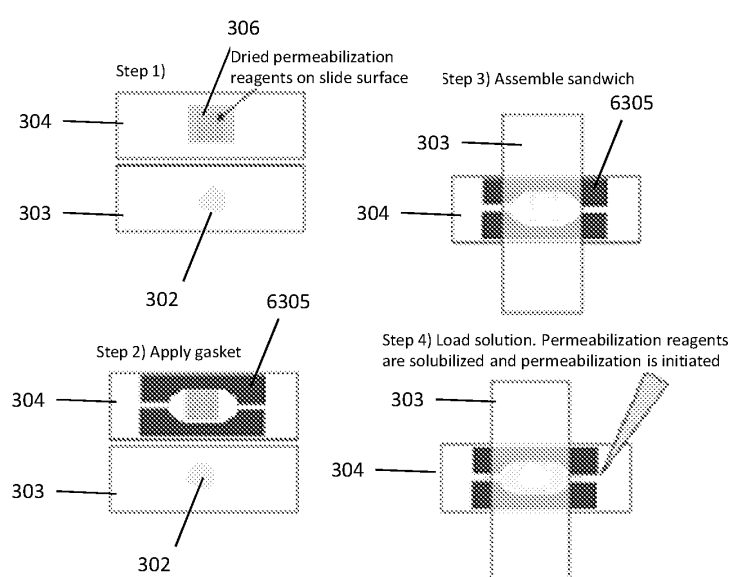
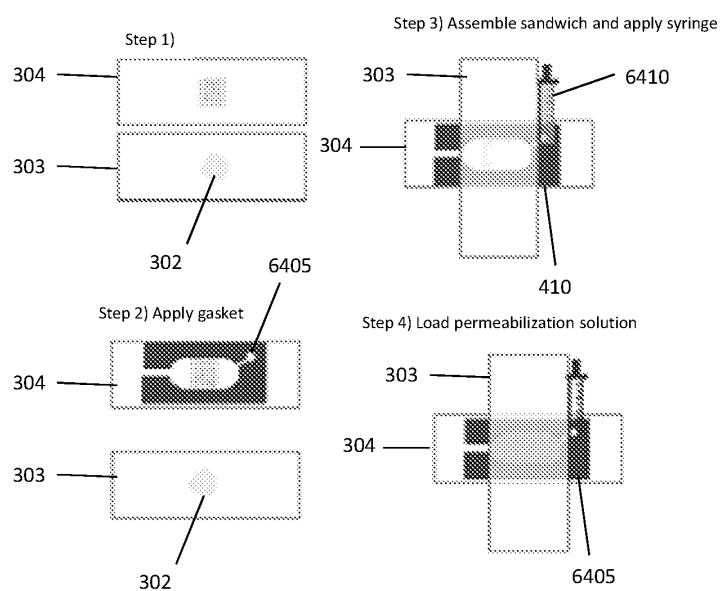


FIG. 62B

**FIG. 63**



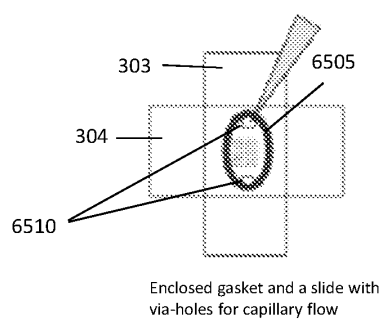


FIG. 65A

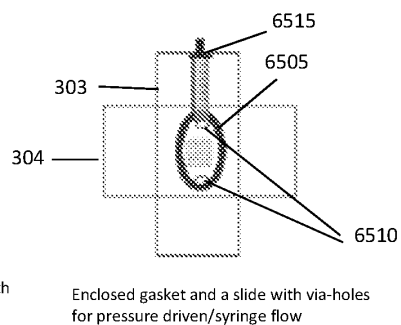
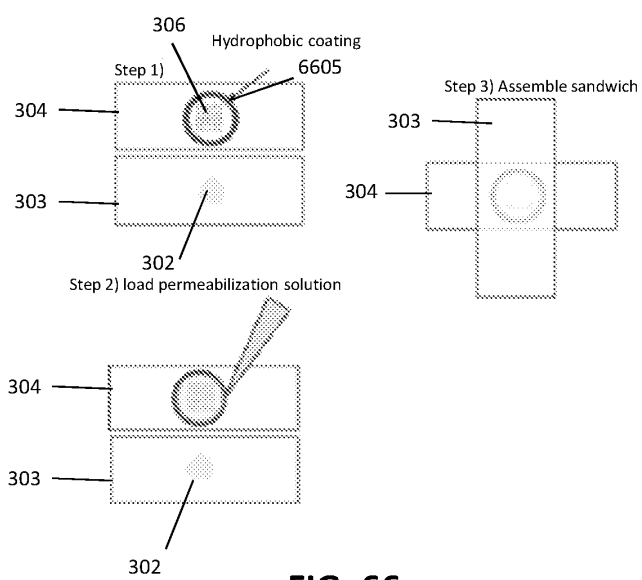


FIG. 65B

**FIG. 66**

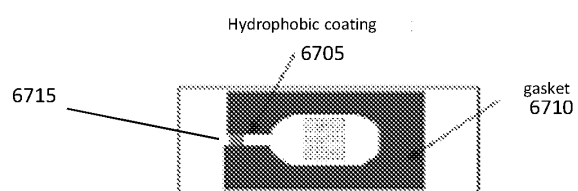


FIG. 67

6800 ↘

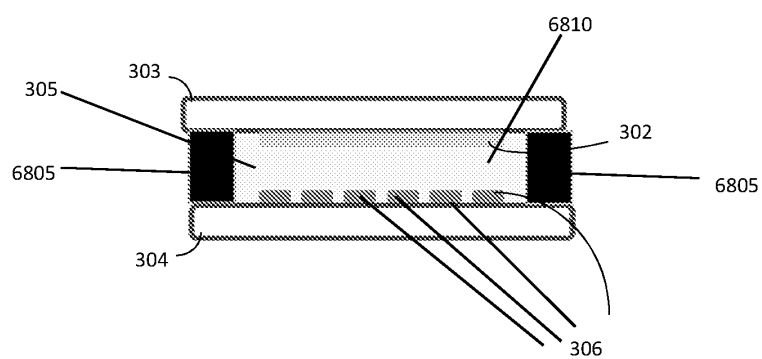


FIG. 68

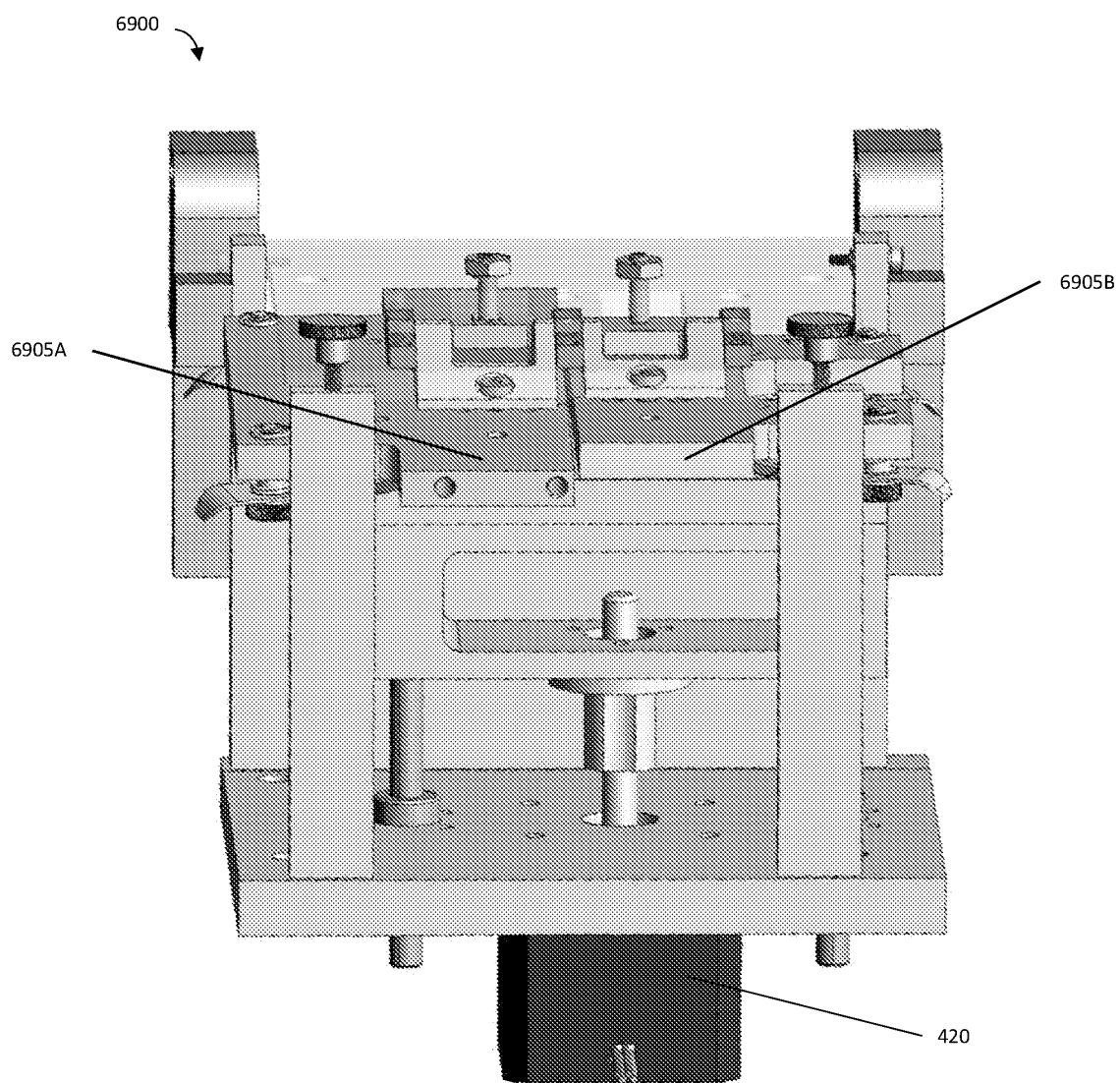


FIG. 69

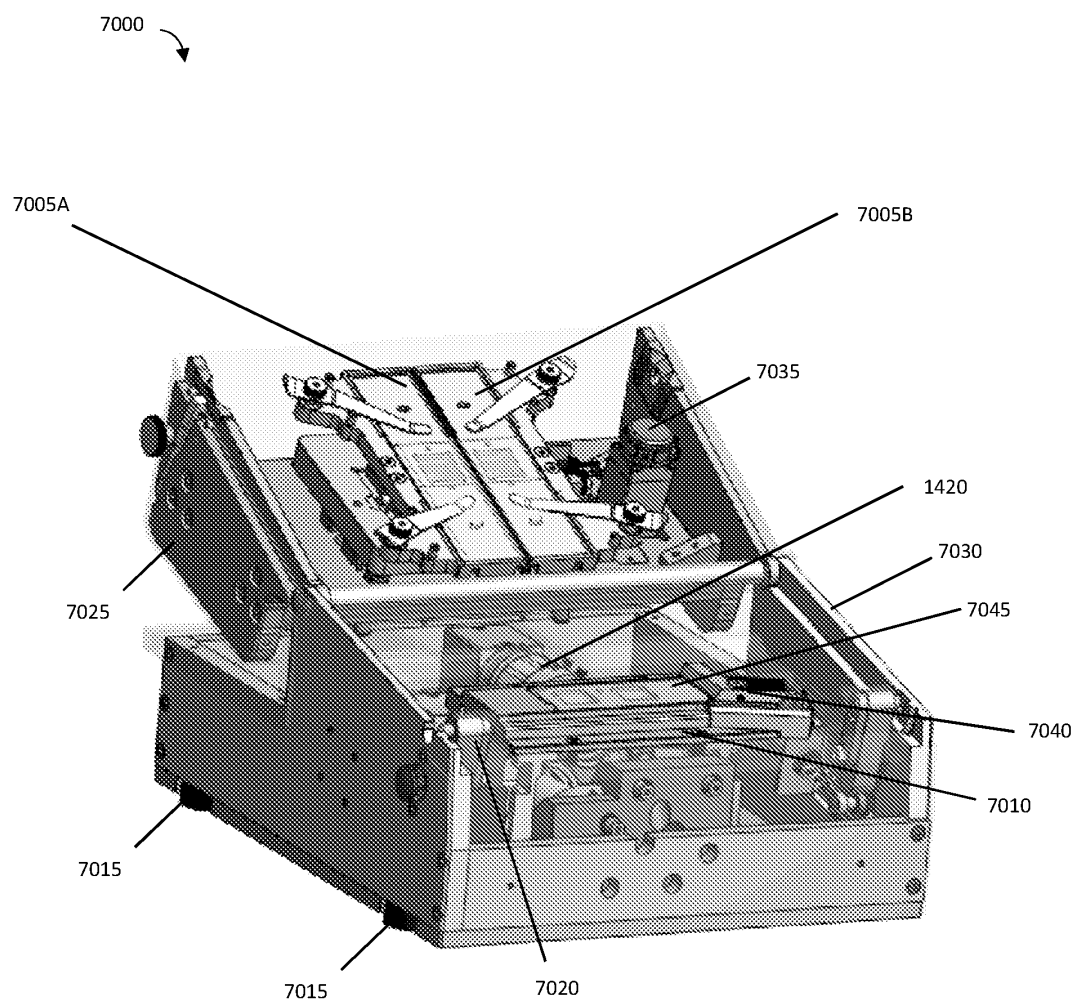


FIG. 70

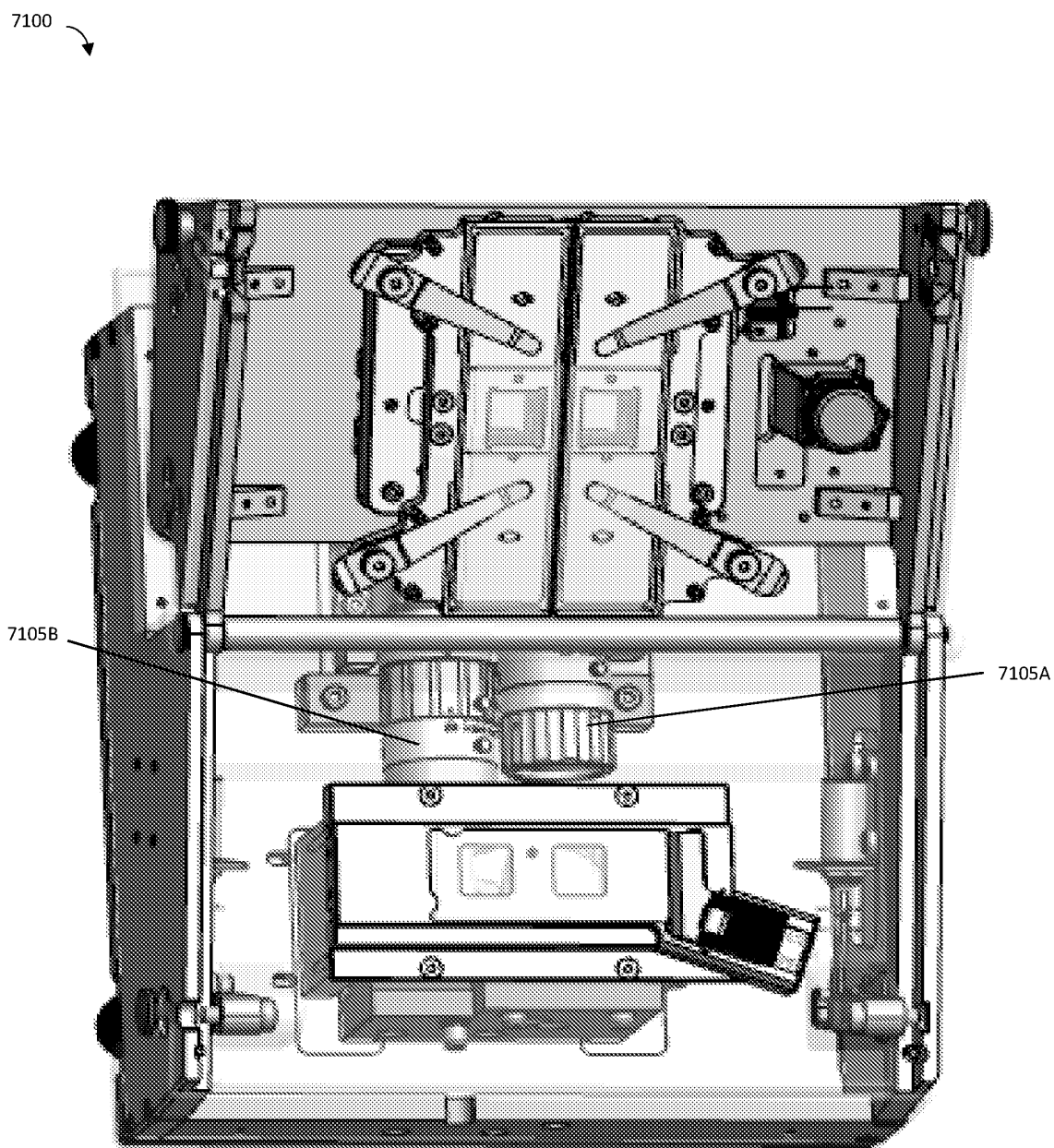


FIG. 71

7200

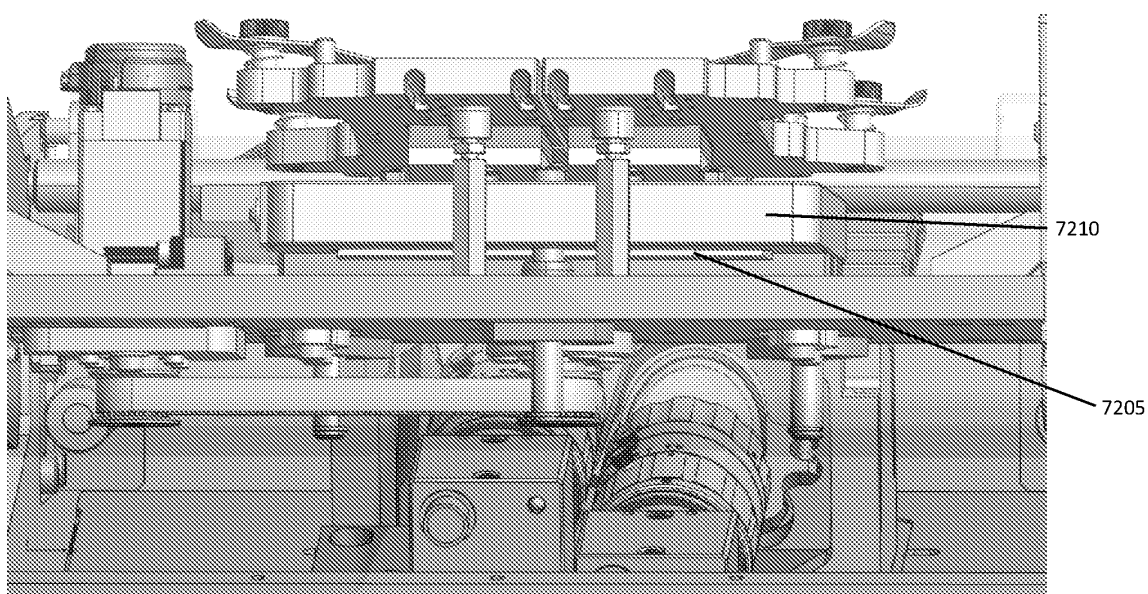


FIG. 72

7300

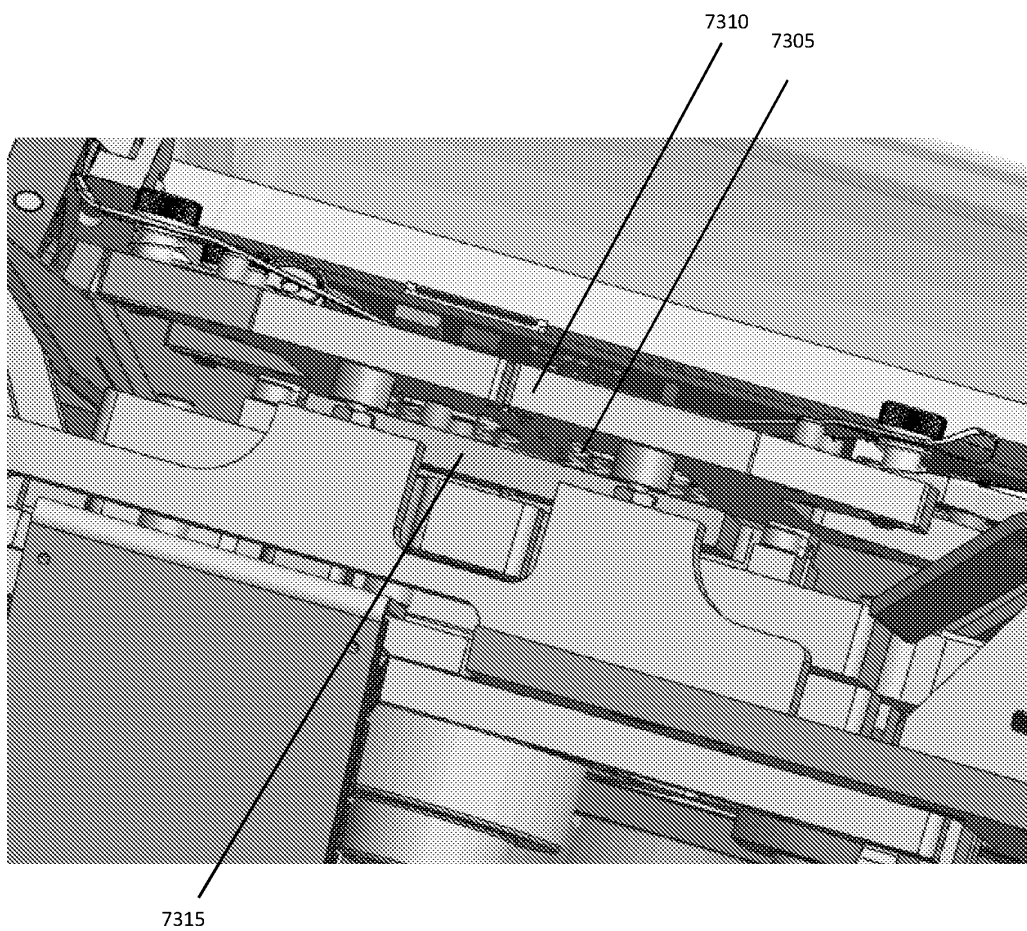


FIG. 73

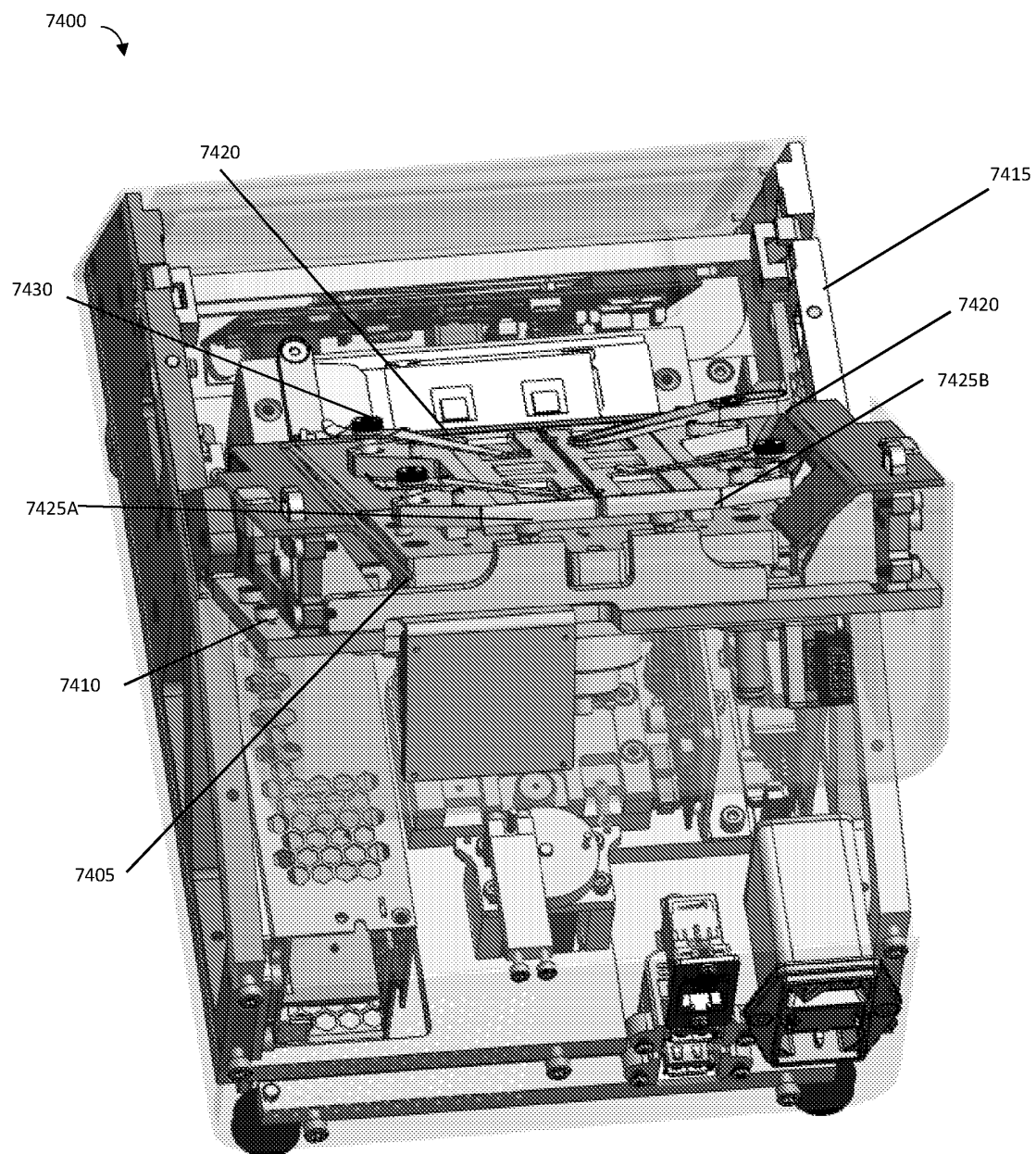


FIG. 74

7500

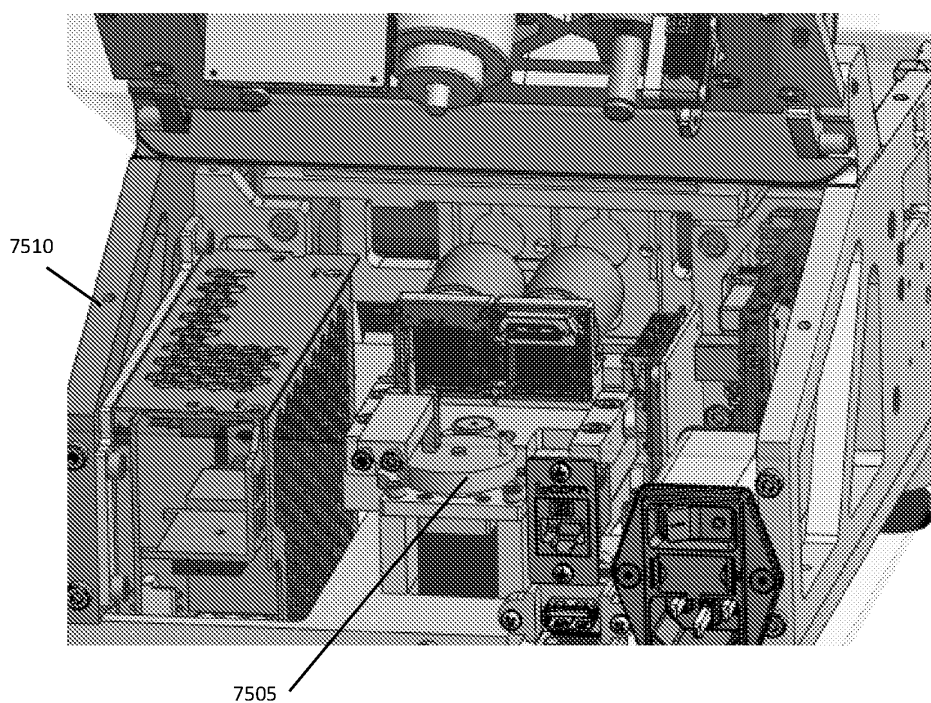



FIG. 75

7600

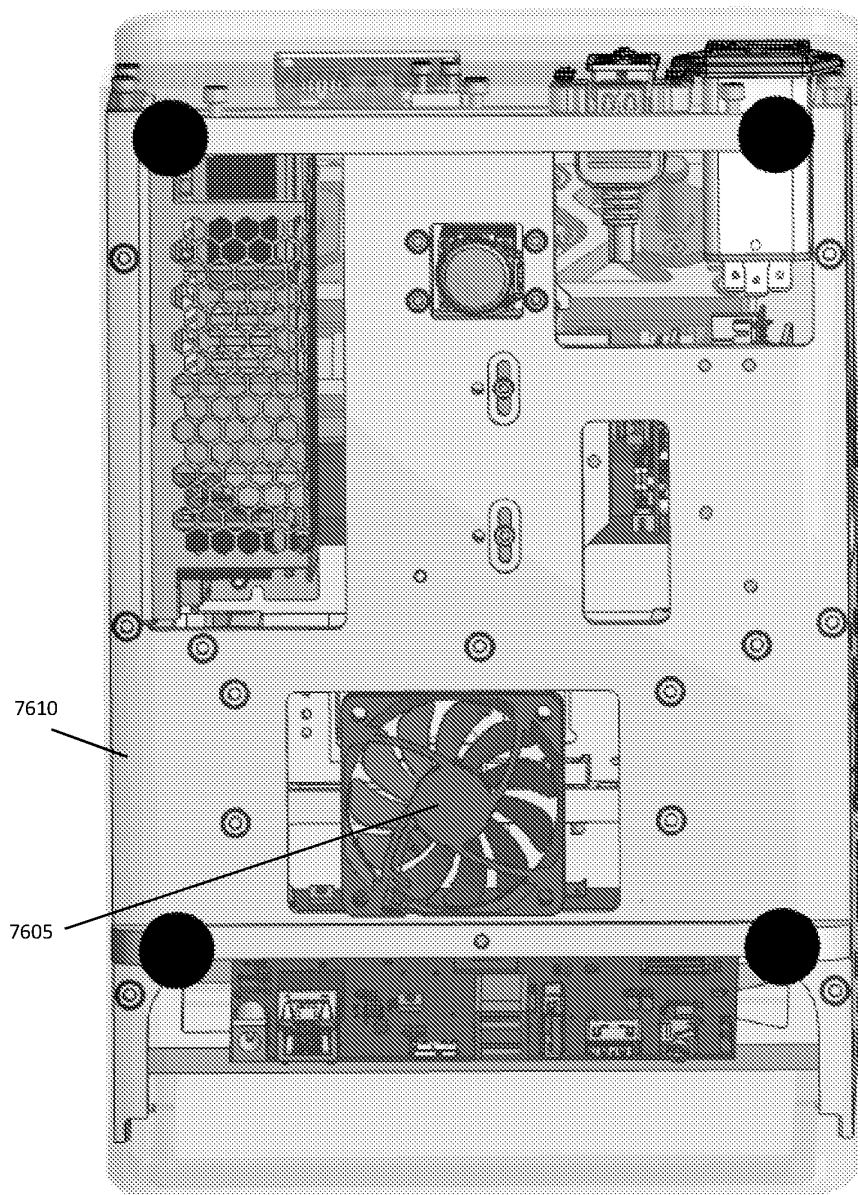


FIG. 76