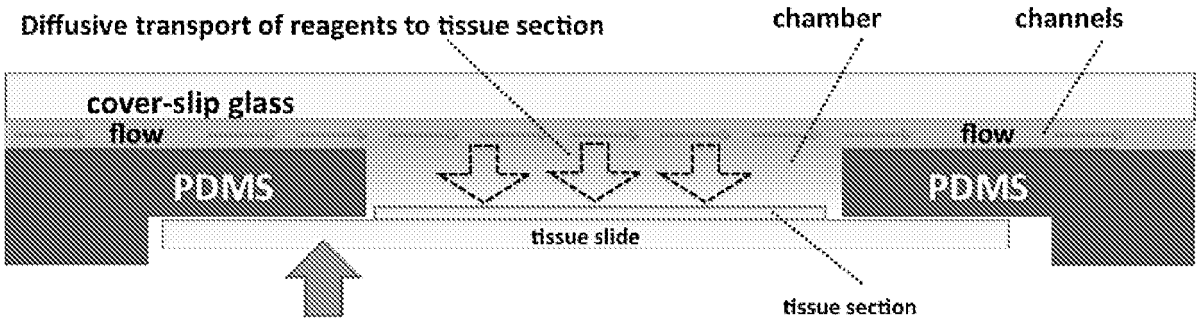


Figure 1

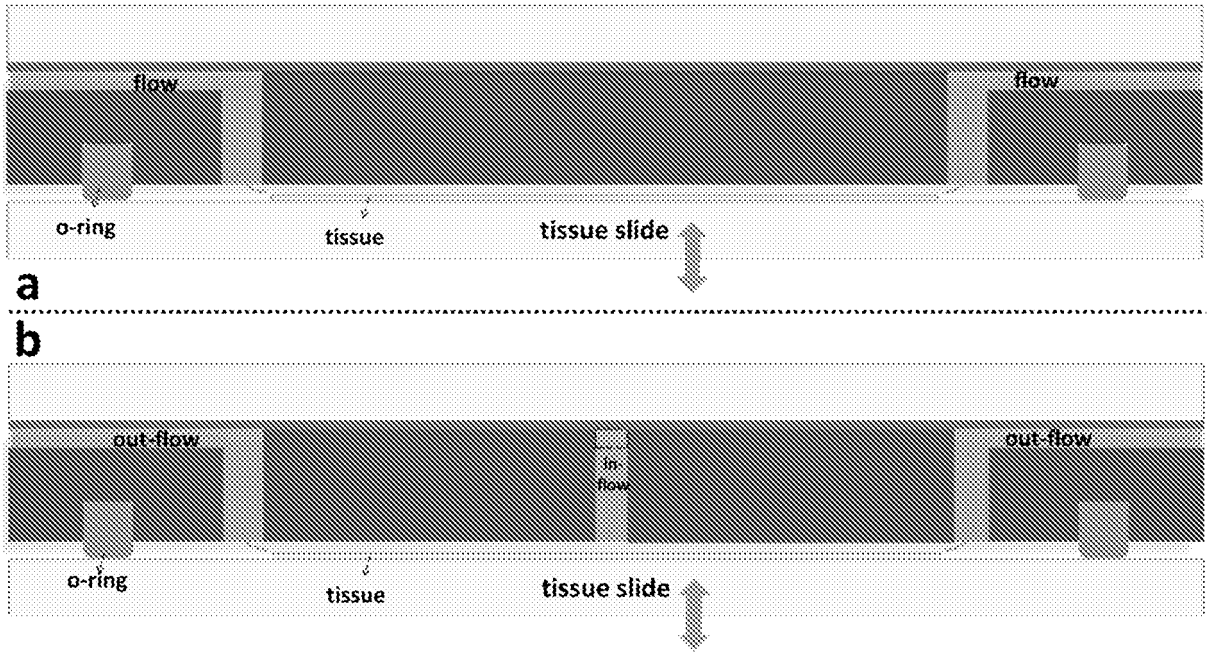


Figure 2

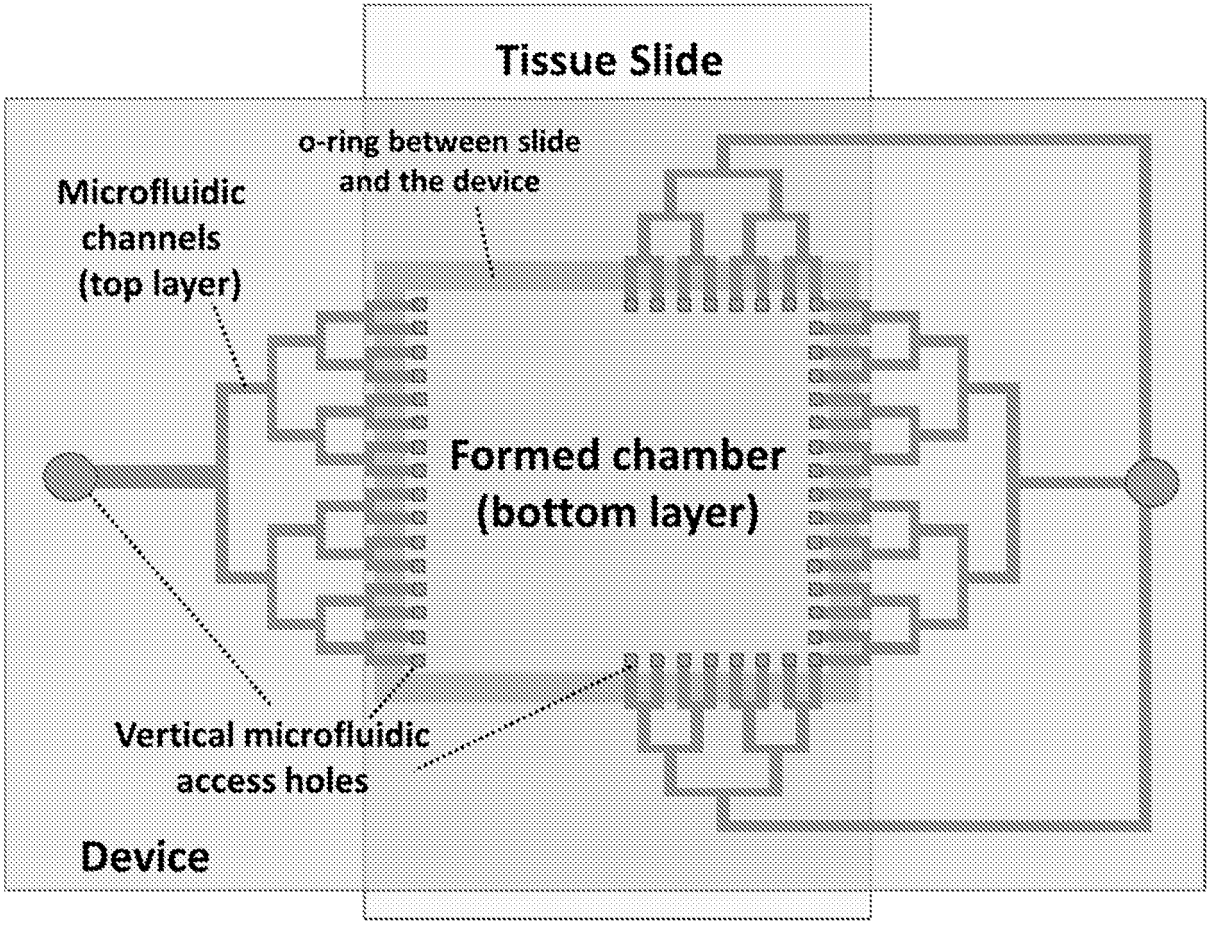


Figure 3

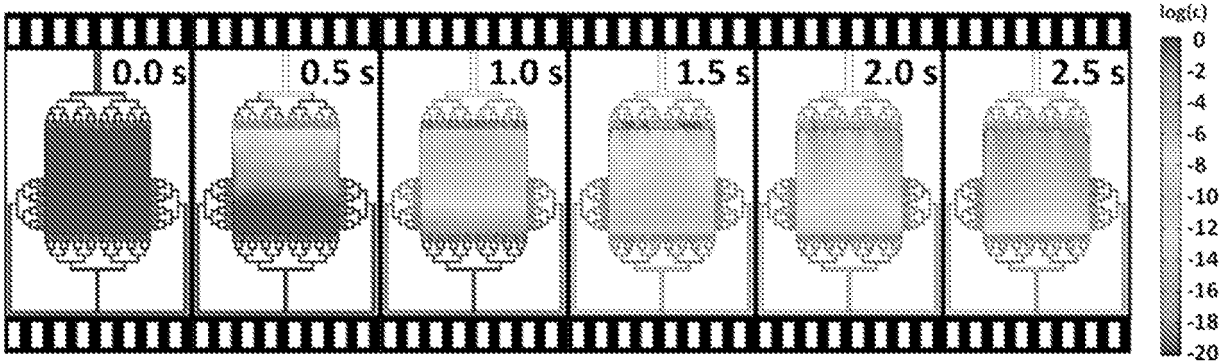


Figure 4

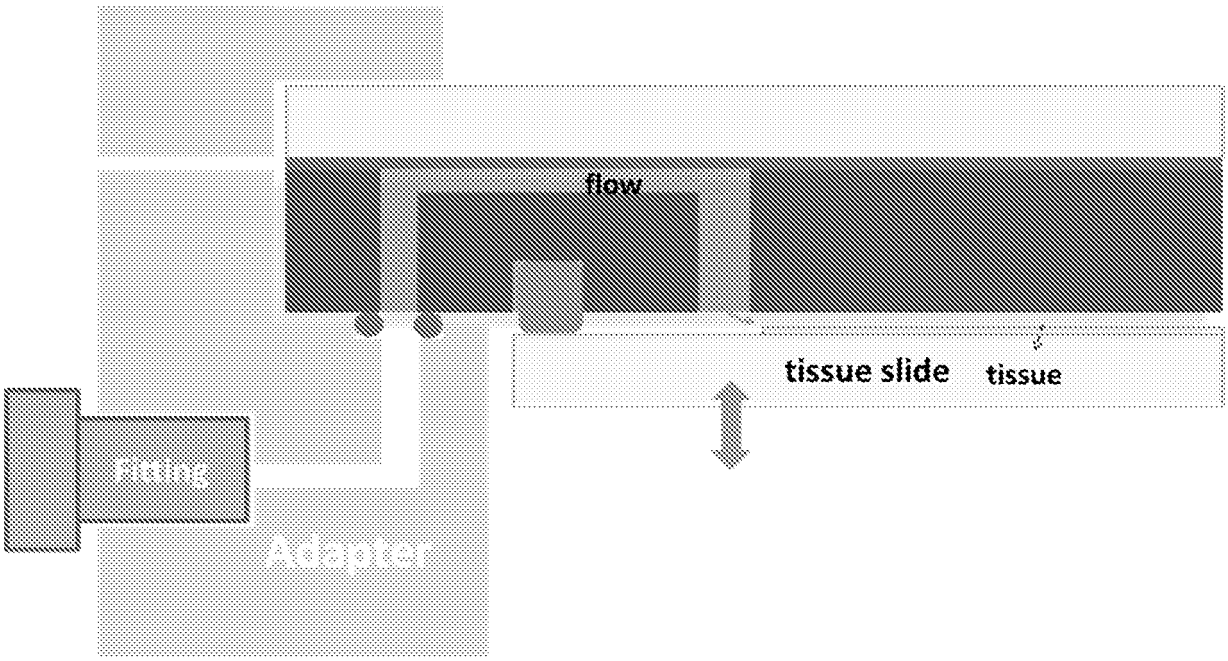


Figure 5

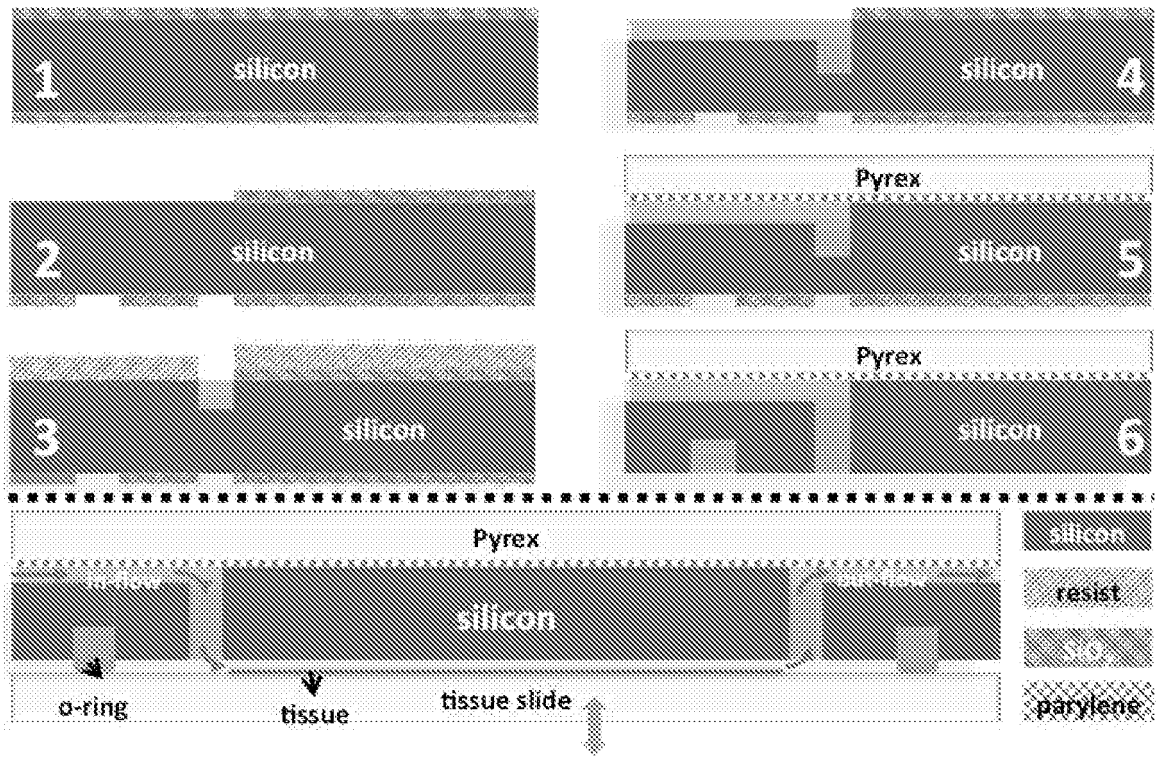


Figure 6

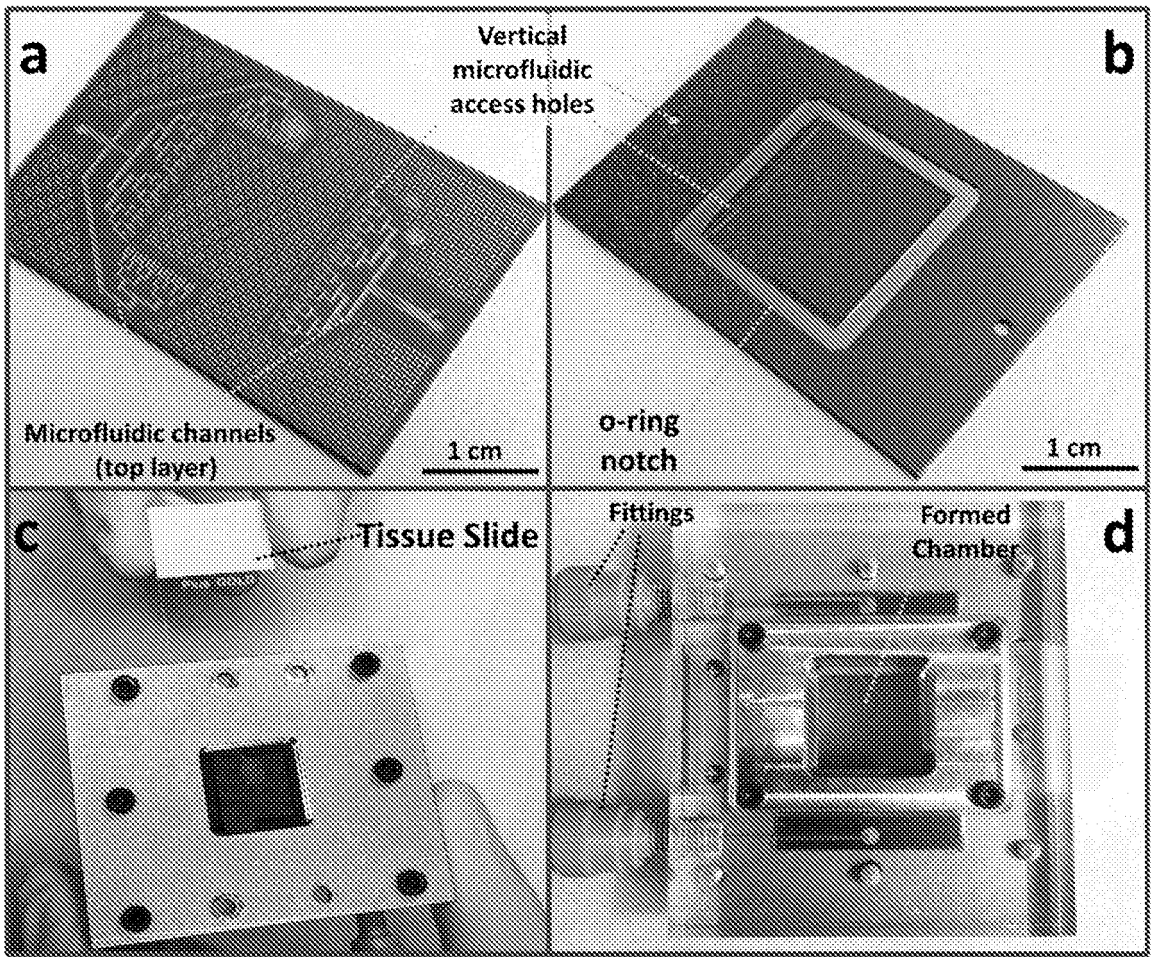

a
Vertical
microfluidic
access holes
b
Microfluidic channels
(top layer)
1 cm
o-ring
notch
1 cm
c
Tissue Slide
Fittings
Formed
Chamber
d

Figure 7

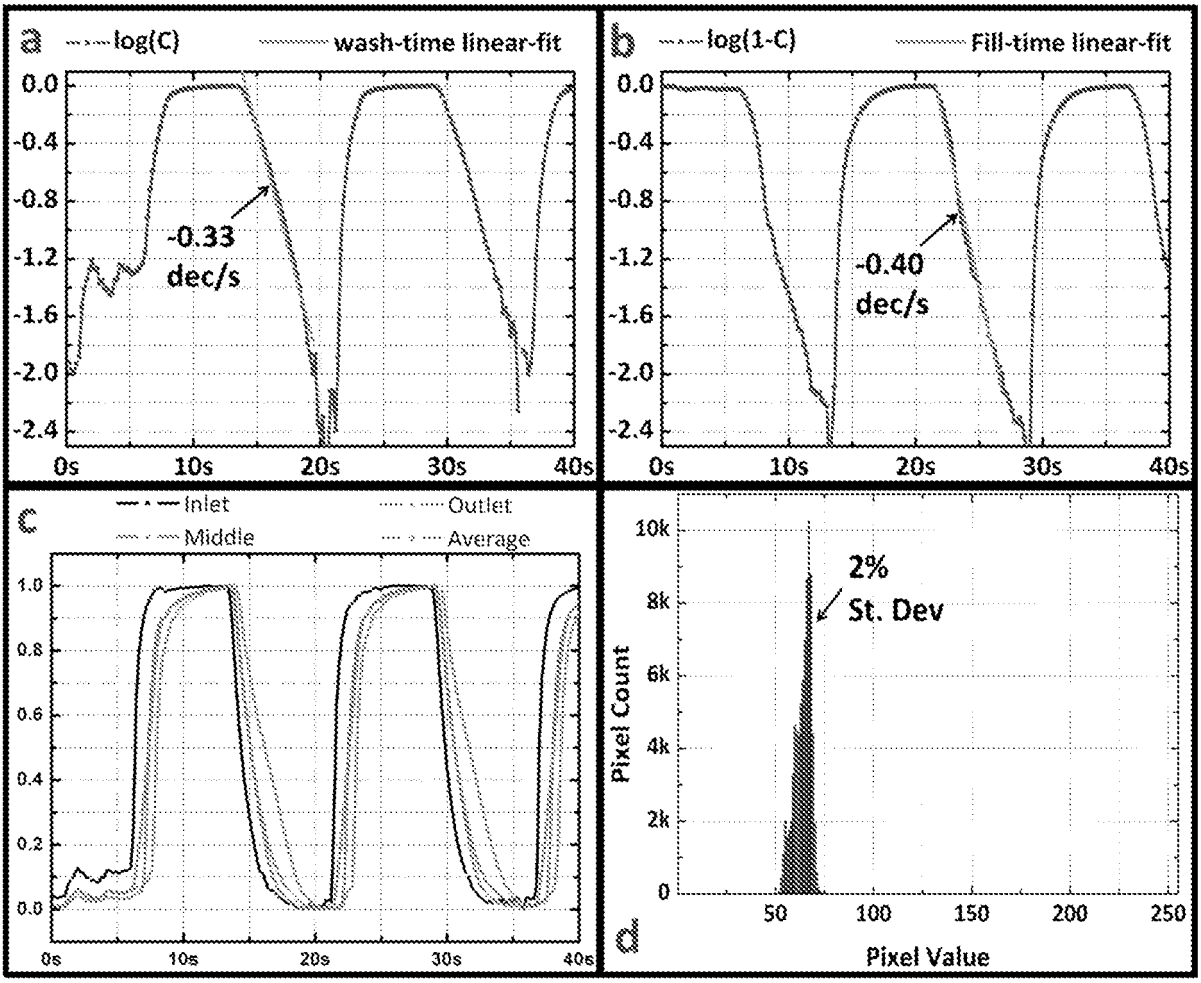

a
log(C)
wash-time linear-fit
0.0
-0.4
-0.8
-1.2
-1.6
-2.0
-2.4
-0.33
dec/s
0s
10s
20s
30s
40s
b
log(1-C)
Fill-time linear-fit
-0.40
dec/s
c
Inlet
Outlet
Middle
Average
1.0
0.8
0.6
0.4
0.2
0.0
d
10k
8k
6k
4k
2k
0
Pixel Count
2%
St. Dev
50
100
150
200
250
Pixel Value

Figure 8

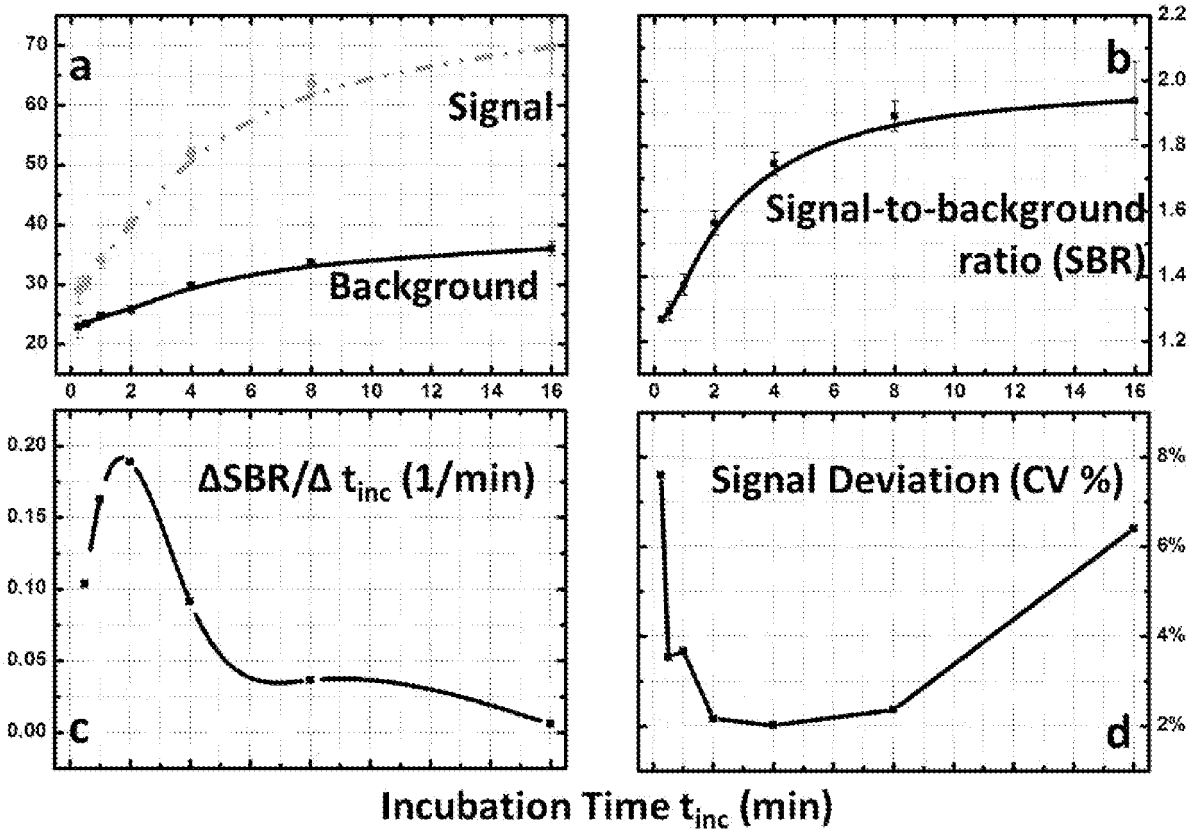

a
Signal
Background
b
Signal-to-background ratio (SBR)
ΔSBR/Δ t_inc (1/min)
c
Signal Deviation (CV %)
d
Incubation Time t_inc (min)

Figure 9

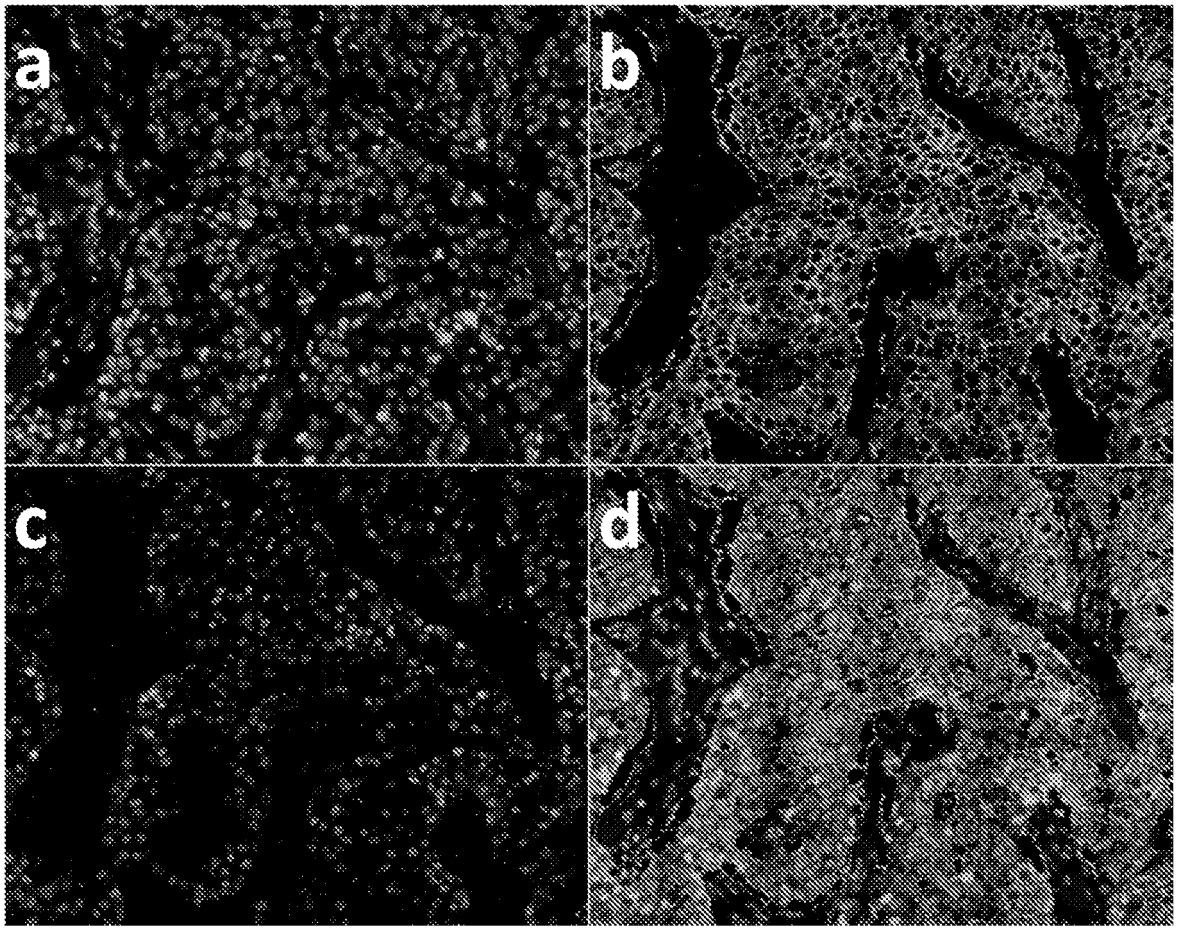
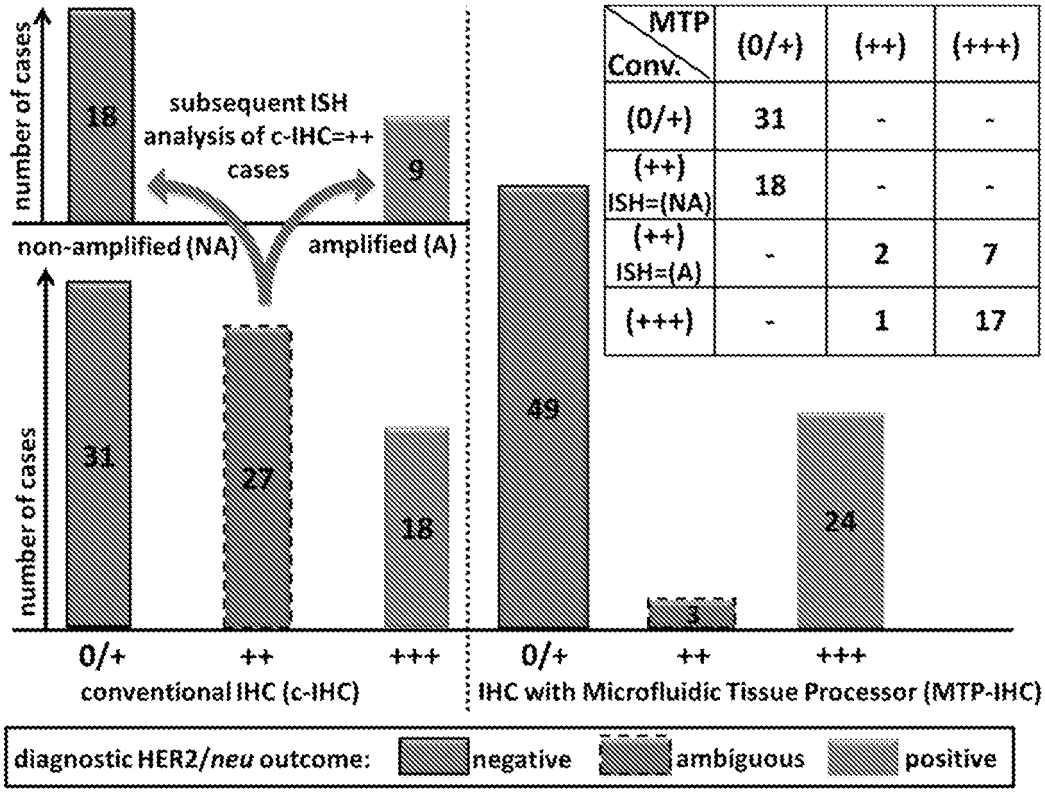


Figure 10



MTP / Conv.	(0/+)	(++)	(+++)
(0/+)	31	-	-
(++) ISH=(NA)	18	-	-
(++) ISH=(A)	-	2	7
(+++)	-	1	17

Figure 11

Tables

Table 1 - Timing of the IHC assay applied on the microfluidic device for incubation time optimization studies. prAb and sdAb corresponds to primary and secondary antibody, respectively. For each biomarker, different incubation times are studied corresponding to *n = -2,-1, 0, 1, 2, 3, 4*

Reagent	Flow duration (s)	Incubation time (min)	Step time (s)
Buffer	7	-	7
prAb	5	2^n	$60(2^n)+5$
Buffer	7	-	7
sdAb with fluorescent label +DAPI	5	2^n	$60(2^n)+5$
Buffer	7	-	-
Total per target	31	2^{n+1}	$60(2^{n+1})+31$

Table 2 - Timing of a multiplexed IHC assay applied in a microfluidic device

Reagent	Flow duration (s)	Incubation time (s)	Step time (s)
Buffer	7	-	7
$prAb_1$ + $prAb_2$	5	120	125
Buffer	7	-	7
$sdAb_1$ + $sdAb_2$ with fluorescent label	5	120	125
Buffer	7	7	7
Total	31	240	**271**
Total per target without neg. cont.	16	120	**136**

Table 3 – Provisional timing of IHC pre-processing of FFPE TSs applied in a microfluidic device

Reagent	Flow duration (s)	Incubation or heating (s)	Step time (s)
Dewaxing (Xylene flow)	30	-	7
Rehydration (DI & Ethanol)	60		65
Antigen retrieval solution and heating	15	45	7
Antigen retrieval	-	120	65
Buffer	30	-	-
Total	135	165	**300**

Table 4 – Comparison of conventional IHC assay and previously demonstrated lab-on-a-chips with the present system

System	Slide Type	Stained area per target	Reagent (µL)	Reagent Unit Cost	Minimum time per target (min)	Figure of merit Area/(vol*min*cost)
Conventional	Standard	100%	1000	2	120	4.15
Our 1st generation	Standard	38%	33	10	20	52.34
MMIHC	Standard	1.5%	30-60	0.1	90 to 154	55
"The wave"	Standard	100%	250 to 350	2	15	132.5
Our 2nd generation	Non-Standard	100%	180	10	3½	158
This system non-mutiplexed	Standard	**100%**	**200**	**1**	**2½**	**2000**
This system multiplexed	Standard	**100%**	**100**	**1**	**1¼**	**8000**