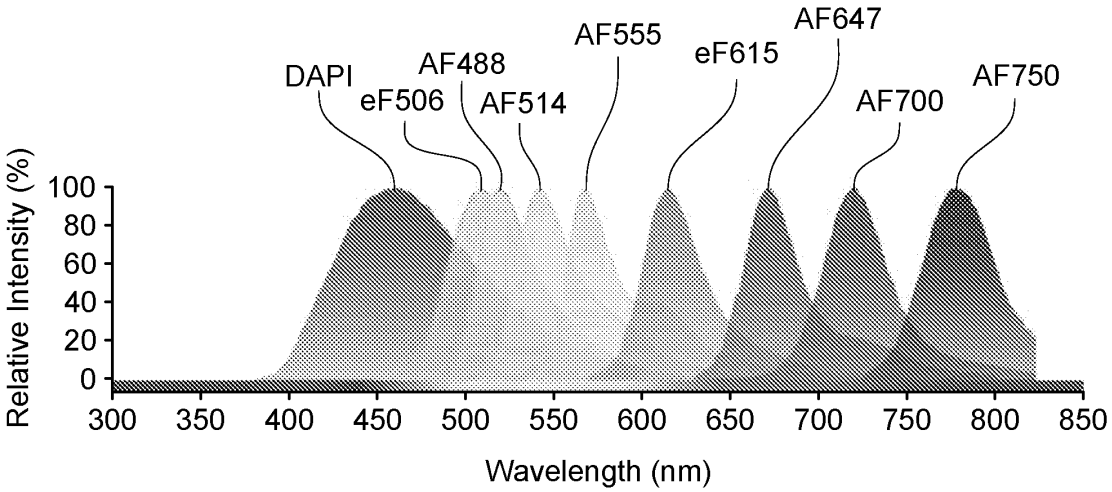

DAPI
eF506
AF488
AF514
AF555
eF615
AF647
AF700
AF750
Relative Intensity (%)
100
80
60
40
20
0
300
350
400
450
500
550
600
650
700
750
800
850
Wavelength (nm)

FIG. 1A

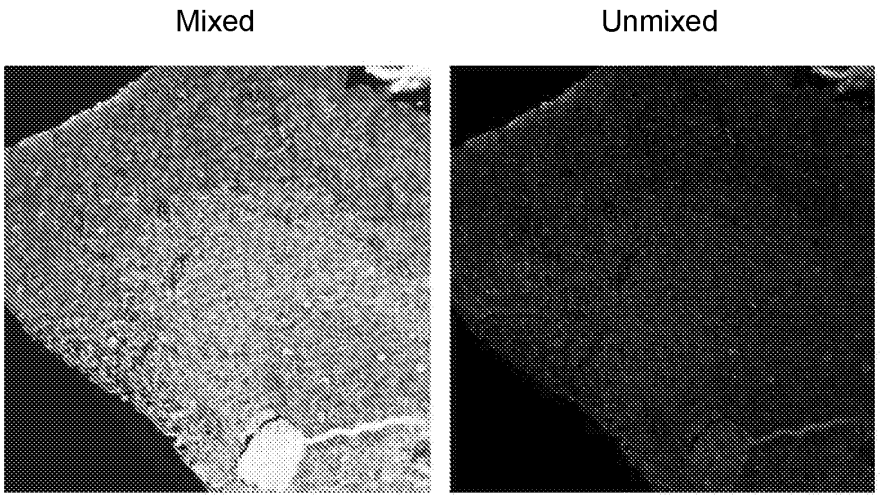

Mixed
Unmixed

FIG. 1B

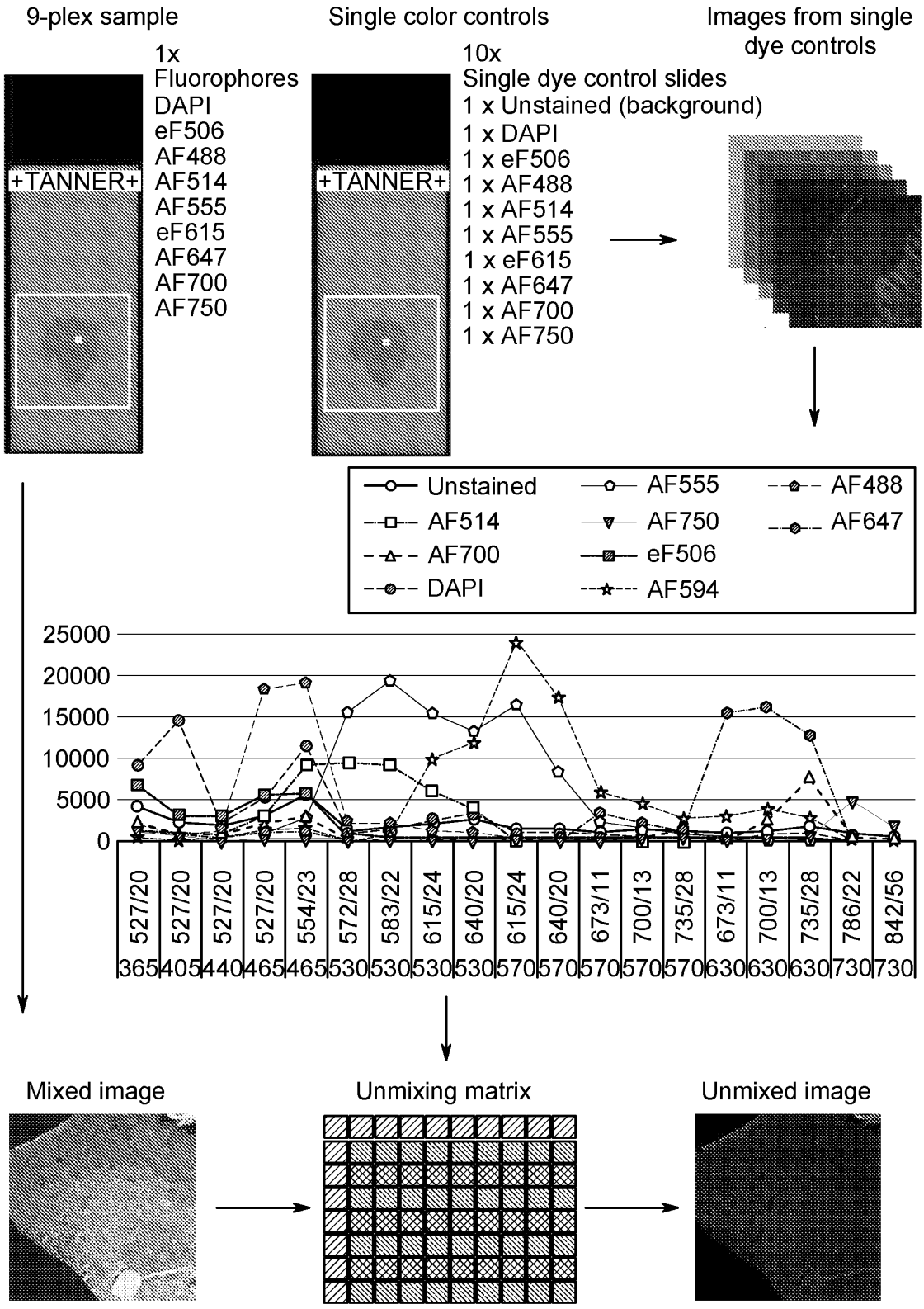

9-plex sample
1x
Fluorophores
DAPI
eF506
AF488
AF514
AF555
eF615
AF647
AF700
AF750
+TANNER+
Single color controls
10x
Single dye control slides
1 x Unstained (background)
1 x DAPI
1 x eF506
1 x AF488
1 x AF514
1 x AF555
1 x eF615
1 x AF647
1 x AF700
1 x AF750
+TANNER+
Images from single dye controls
Unstained
AF555
AF488
AF514
AF750
AF647
AF700
eF506
DAPI
AF594
25000
20000
15000
10000
5000
0
527/20 365
527/20 405
527/20 440
527/20 465
554/23 465
572/28 530
583/22 530
615/24 530
640/20 530
615/24 570
640/20 570
673/11 570
700/13 570
735/28 570
673/11 630
700/13 630
735/28 630
786/22 730
842/56 730
Mixed image
Unmixing matrix
Unmixed image

FIG. 2

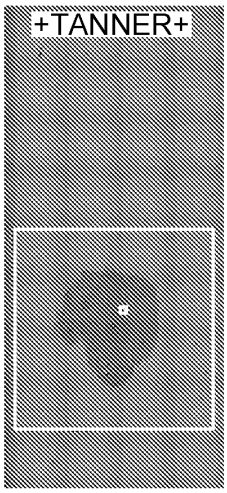


FIG. 3

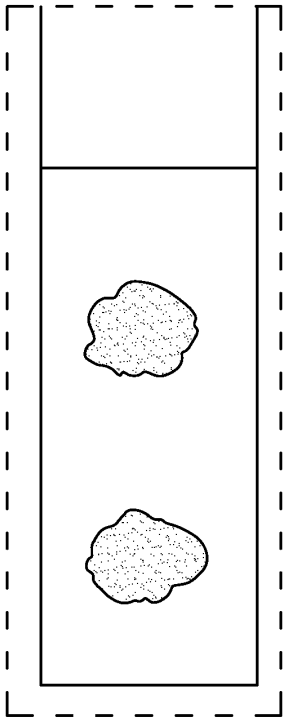


FIG. 4A

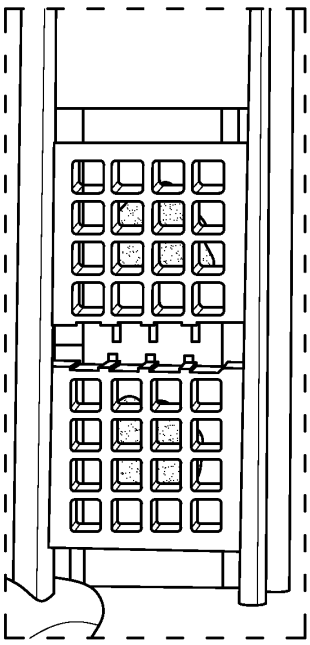


FIG. 4B

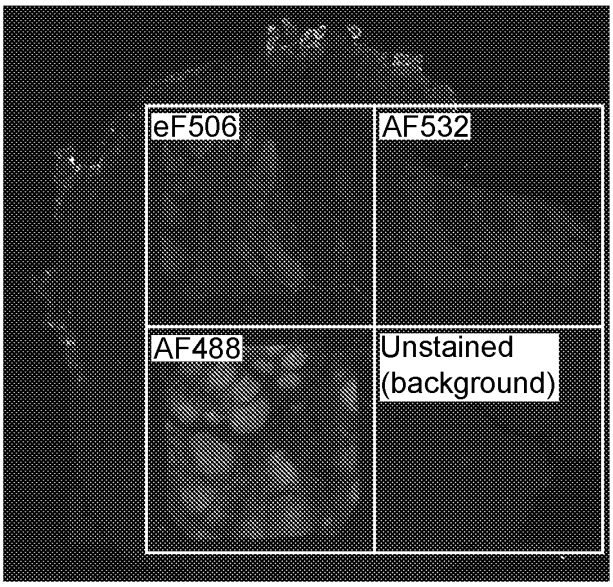

eF506
AF532
AF488
Unstained
(background)

FIG. 5A

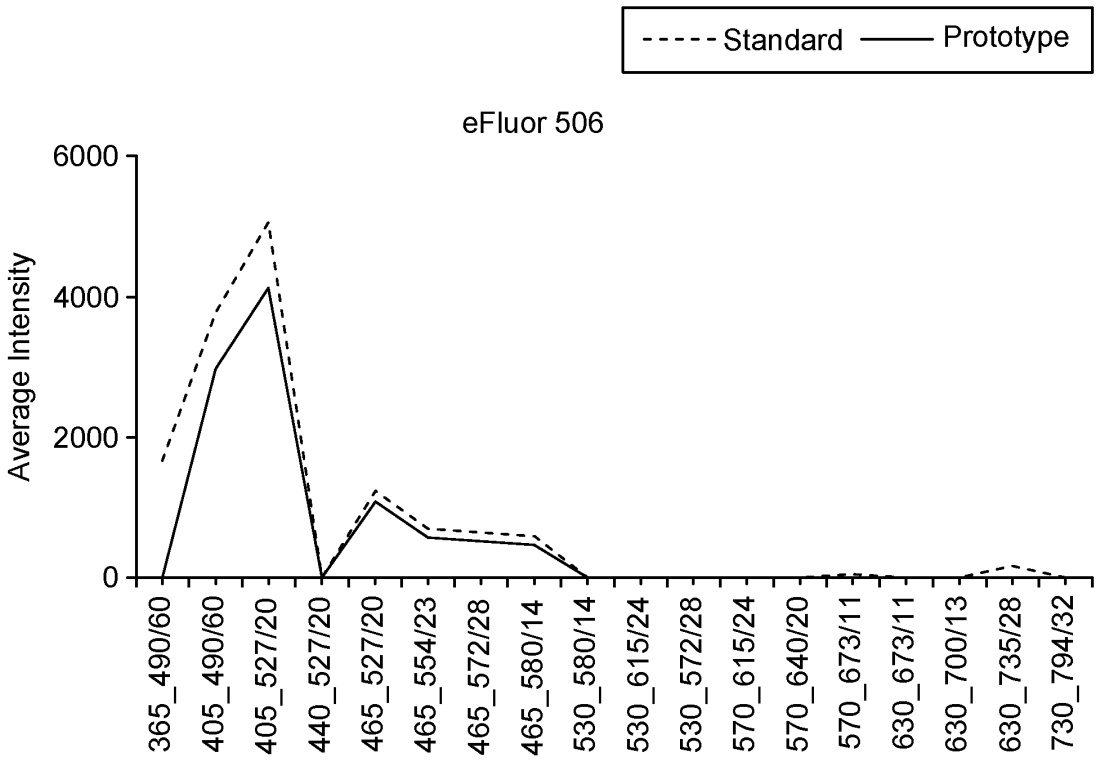

Standard
Prototype
eFluor 506
Average Intensity
6000
4000
2000
0
365_490/60
405_490/60
405_527/20
440_527/20
465_527/20
465_554/23
465_572/28
465_580/14
530_580/14
530_615/24
530_572/28
570_615/24
570_640/20
570_673/11
630_673/11
630_700/13
630_735/28
730_794/32

FIG. 5B

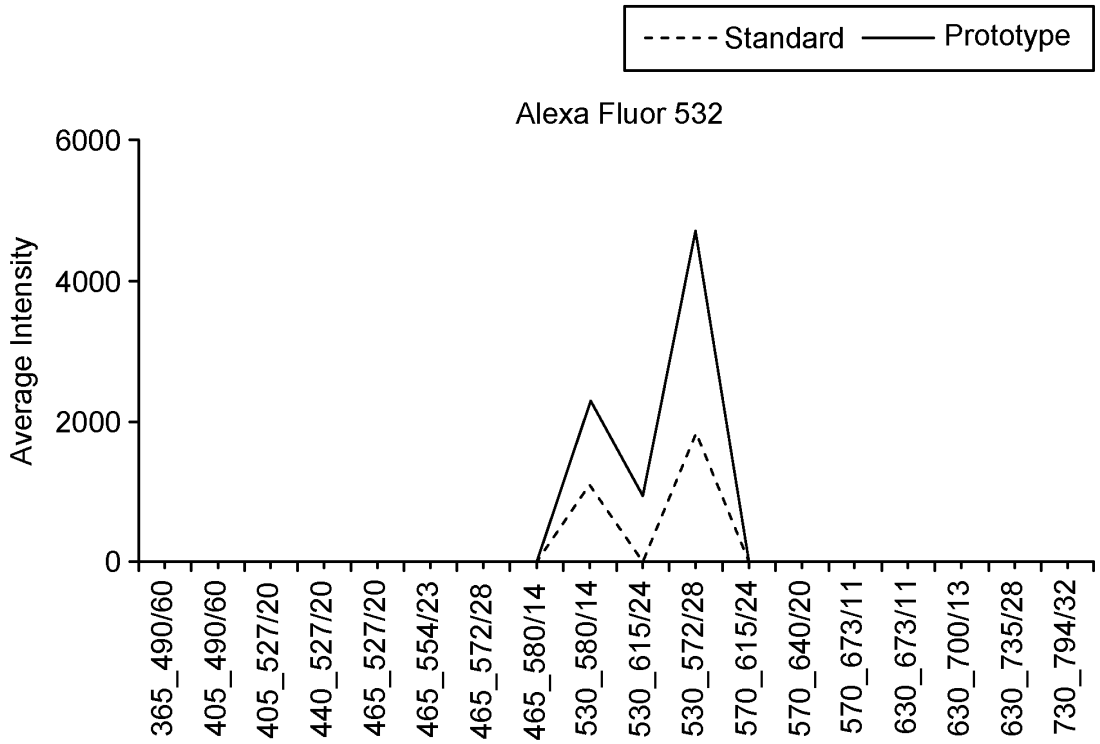

Standard
Prototype
Alexa Fluor 532
Average Intensity
6000
4000
2000
0
365_490/60
405_490/60
405_527/20
440_527/20
465_527/20
465_554/23
465_572/28
465_580/14
530_580/14
530_615/24
530_572/28
570_615/24
570_640/20
570_673/11
630_673/11
630_700/13
630_735/28
730_794/32

FIG. 5C

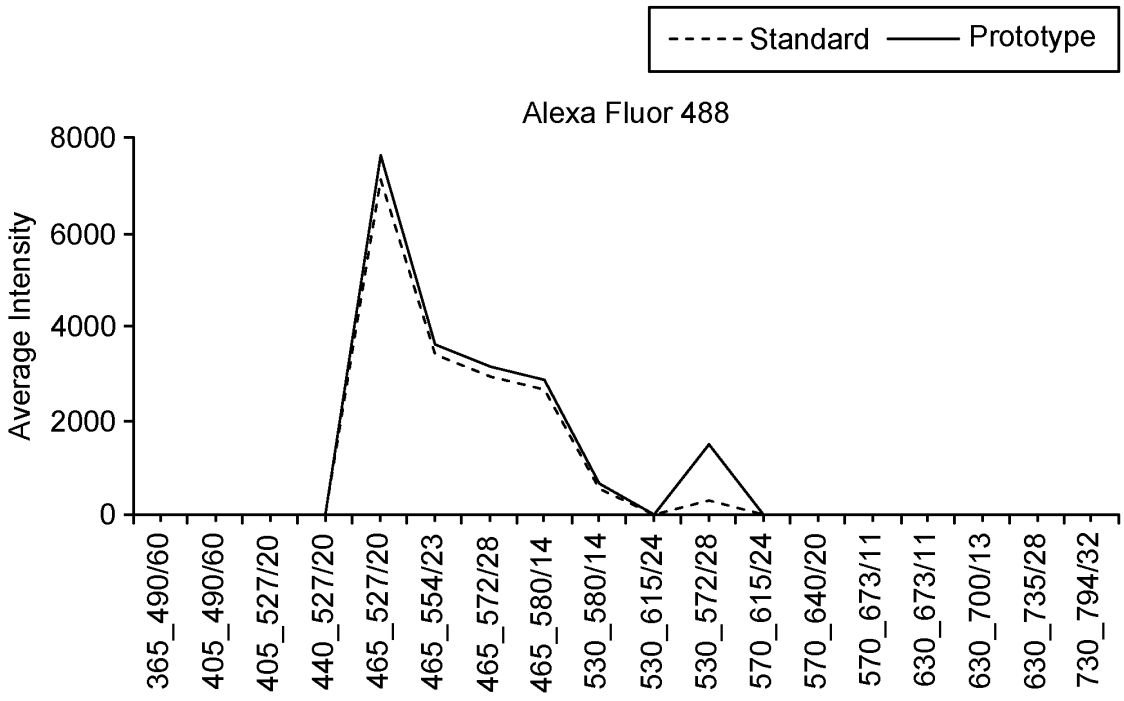

Standard
Prototype
Alexa Fluor 488
Average Intensity
8000
6000
4000
2000
0
365_490/60
405_490/60
405_527/20
440_527/20
465_527/20
465_554/23
465_572/28
465_580/14
530_580/14
530_615/24
530_572/28
570_615/24
570_640/20
570_673/11
630_673/11
630_700/13
630_735/28
730_794/32

FIG. 5D

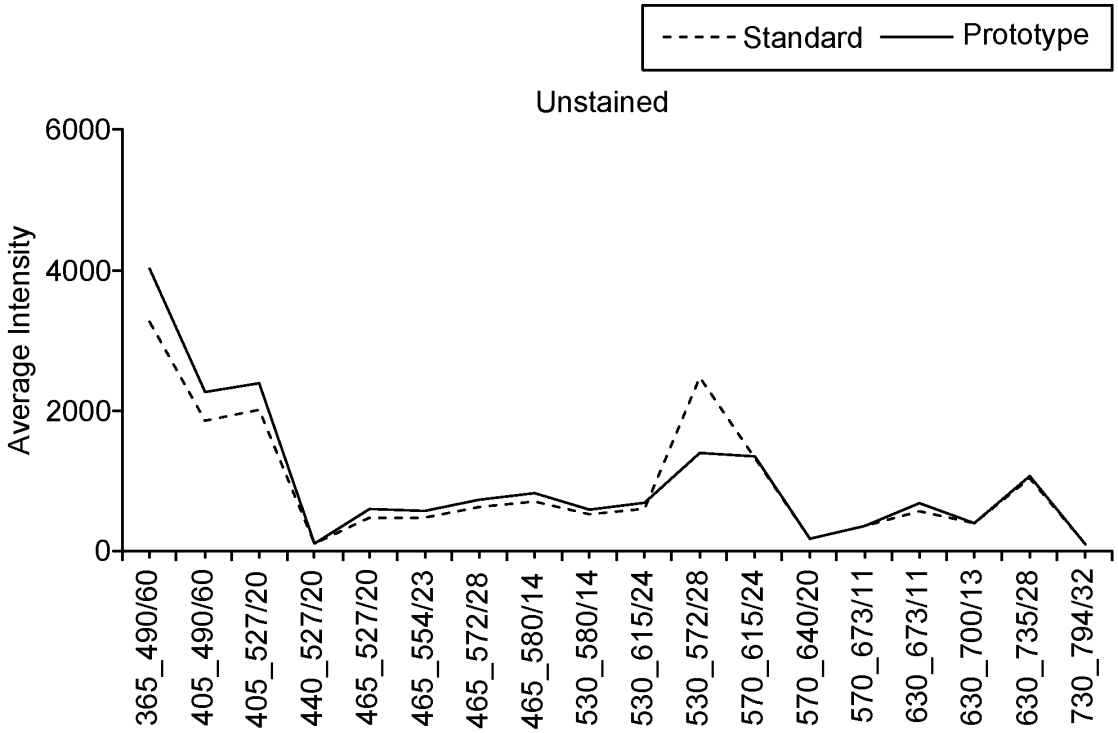

Standard
Prototype
Unstained
Average Intensity
6000
4000
2000
0
365_490/60
405_490/60
405_527/20
440_527/20
465_527/20
465_554/23
465_572/28
465_580/14
530_580/14
530_615/24
530_572/28
570_615/24
570_640/20
570_673/11
630_673/11
630_700/13
630_735/28
730_794/32

FIG. 5E

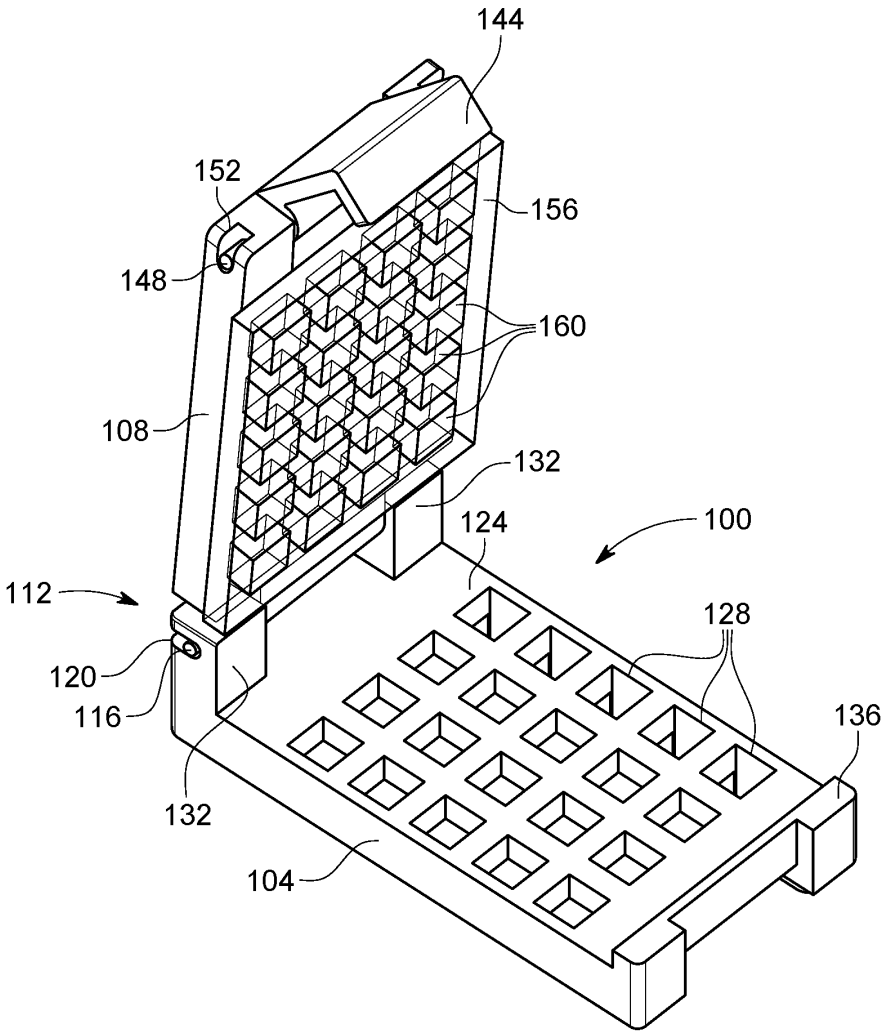


FIG. 6A

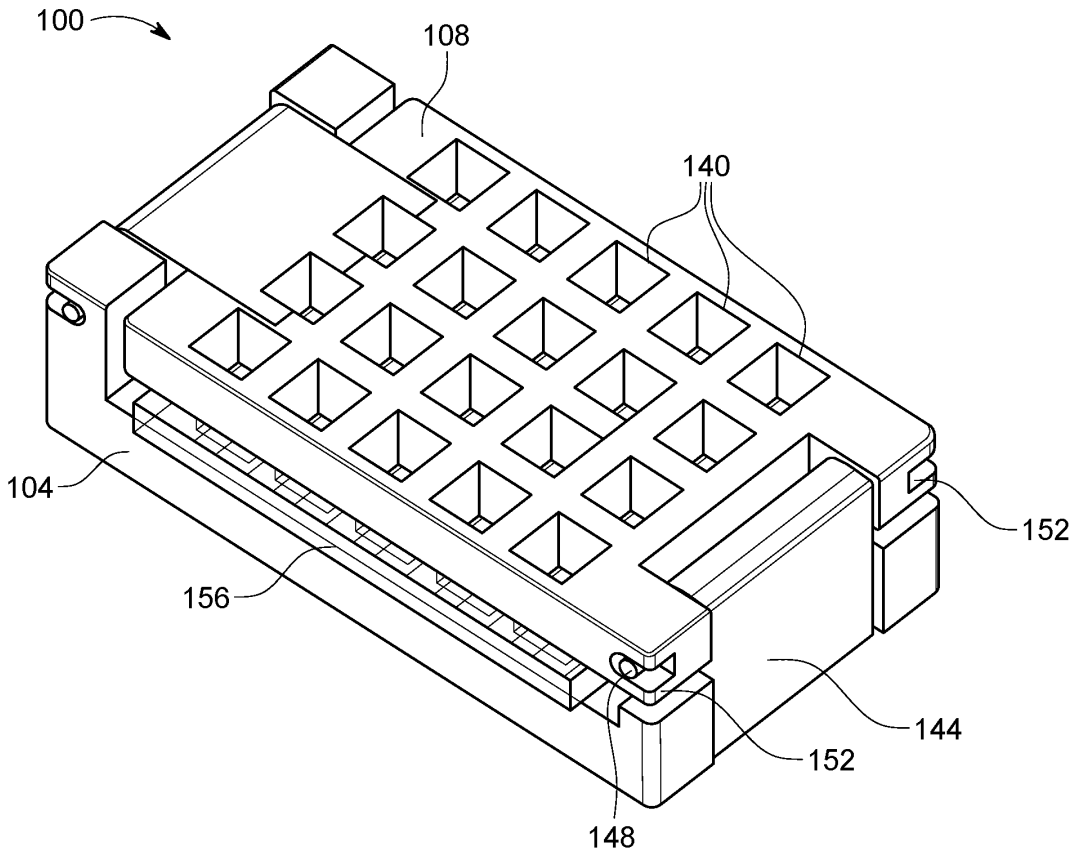


FIG. 6B

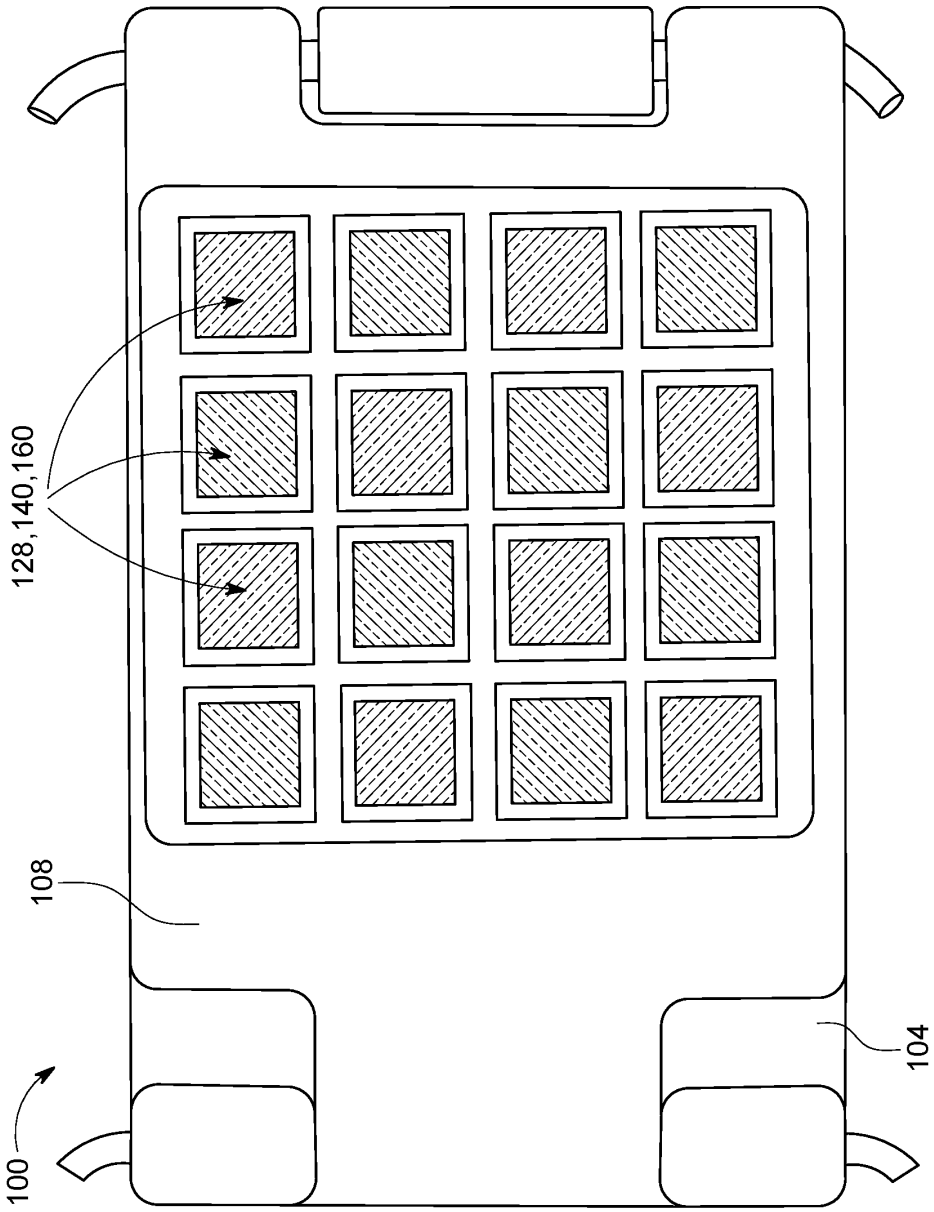


FIG. 6C

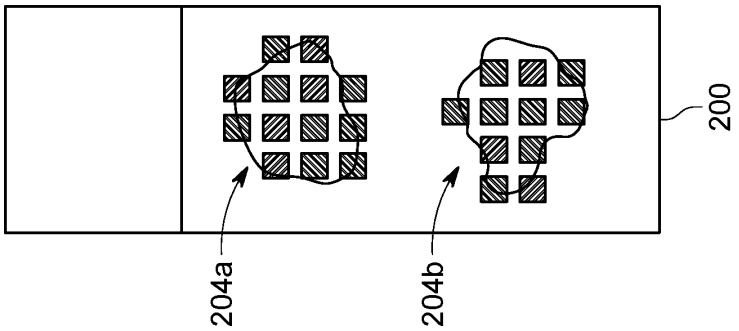


FIG. 7D

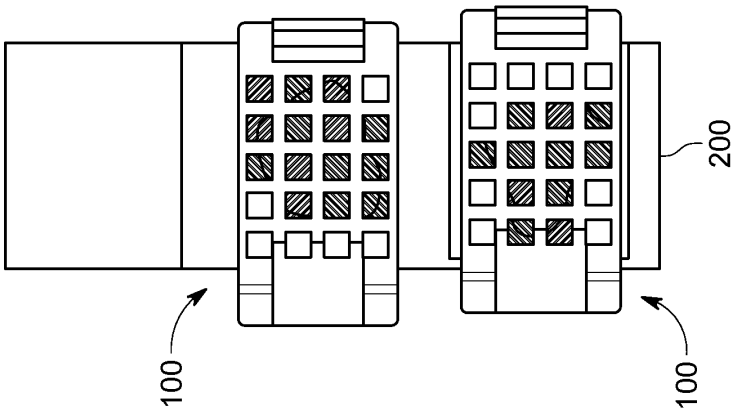


FIG. 7C

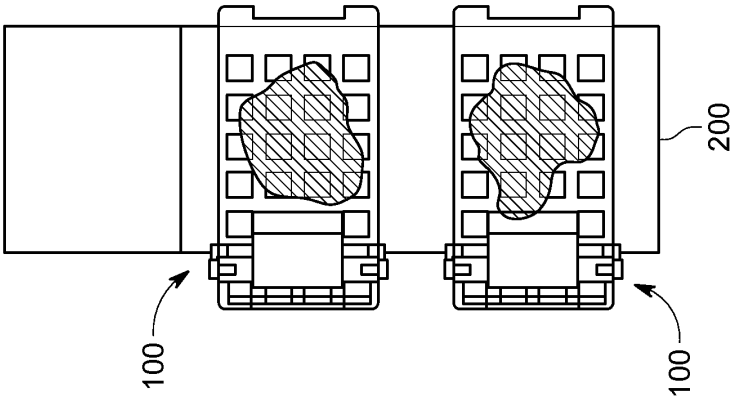


FIG. 7B

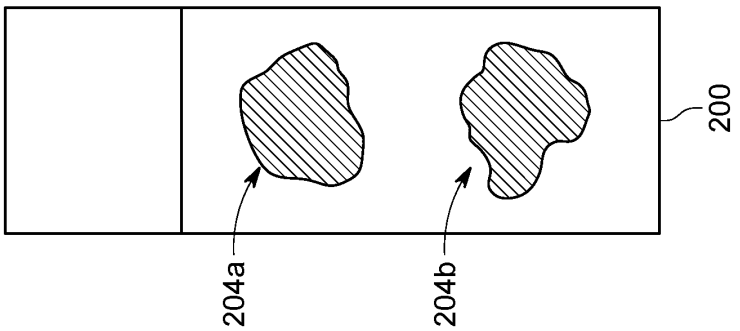


FIG. 7A

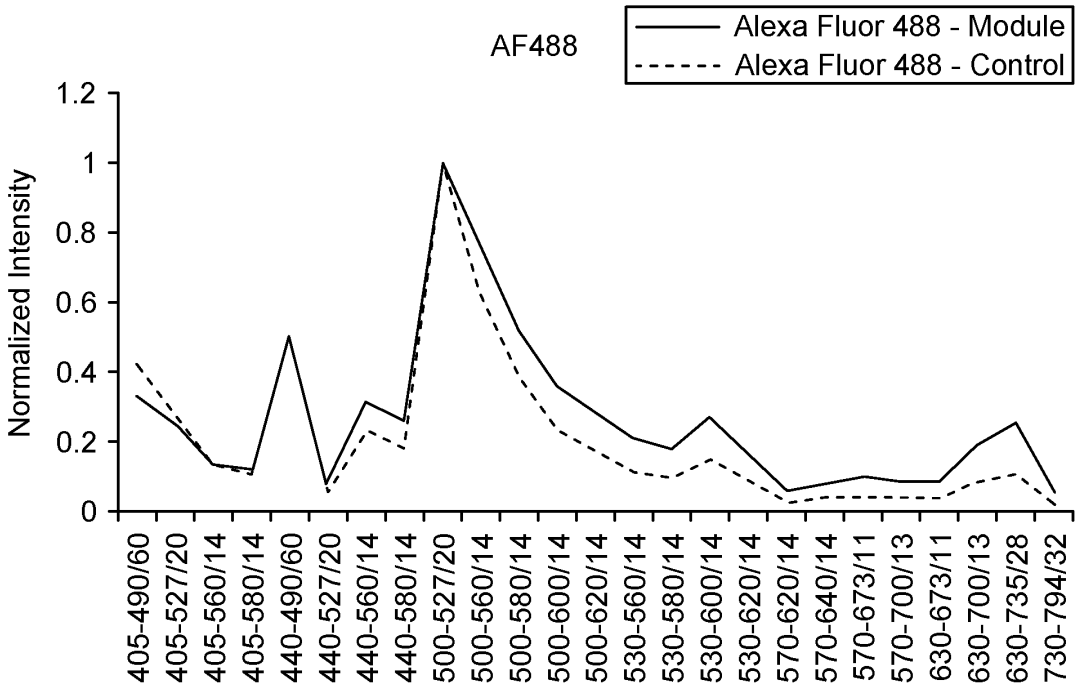

AF488
Alexa Fluor 488 - Module
Alexa Fluor 488 - Control
Normalized Intensity
1.2
1
0.8
0.6
0.4
0.2
0
405-490/60
405-527/20
405-560/14
405-580/14
440-490/60
440-527/20
440-560/14
440-580/14
500-527/20
500-560/14
500-580/14
500-600/14
500-620/14
530-560/14
530-580/14
530-600/14
530-620/14
570-620/14
570-640/14
570-673/11
570-700/13
630-673/11
630-700/13
630-735/28
730-794/32

FIG. 8A

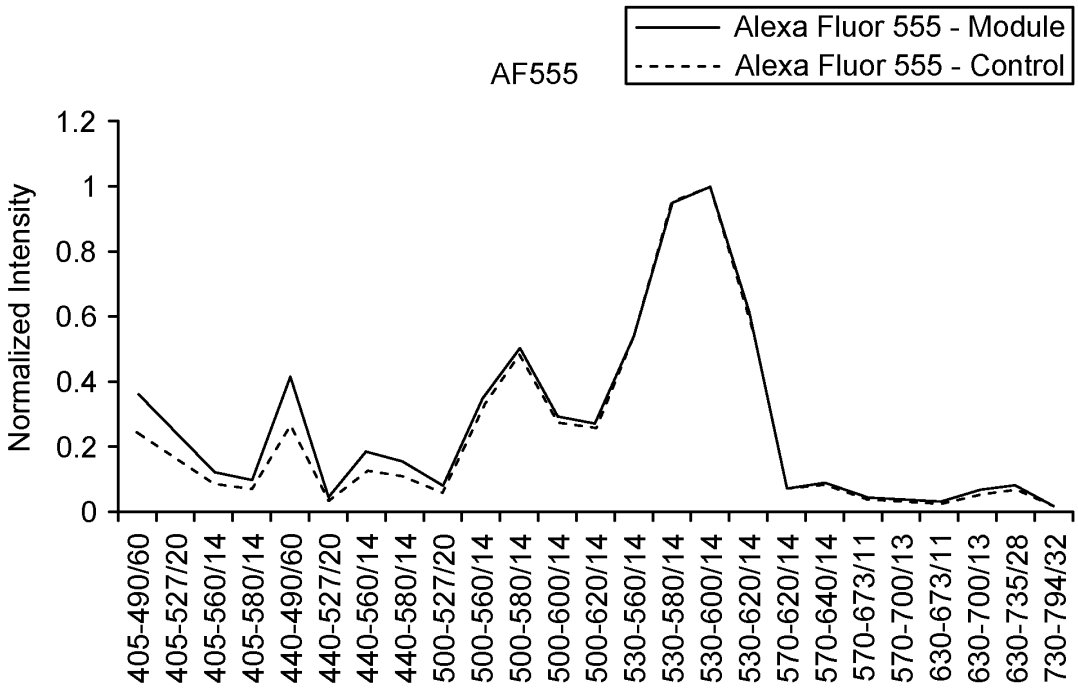

AF555
Alexa Fluor 555 - Module
Alexa Fluor 555 - Control
Normalized Intensity
1.2
1
0.8
0.6
0.4
0.2
0
405-490/60
405-527/20
405-560/14
405-580/14
440-490/60
440-527/20
440-560/14
440-580/14
500-527/20
500-560/14
500-580/14
500-600/14
500-620/14
530-560/14
530-580/14
530-600/14
530-620/14
570-620/14
570-640/14
570-673/11
570-700/13
630-673/11
630-700/13
630-735/28
730-794/32

FIG. 8B

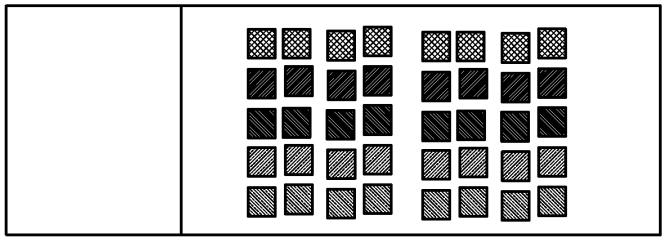


FIG. 9D

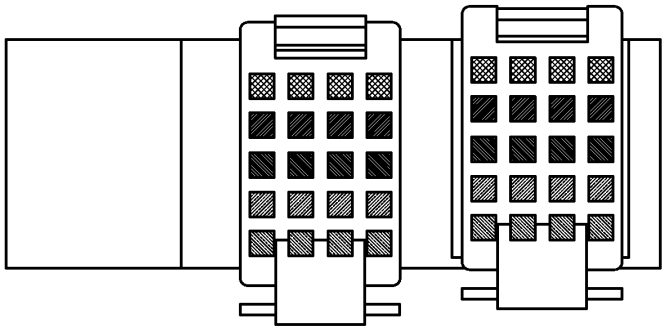


FIG. 9C

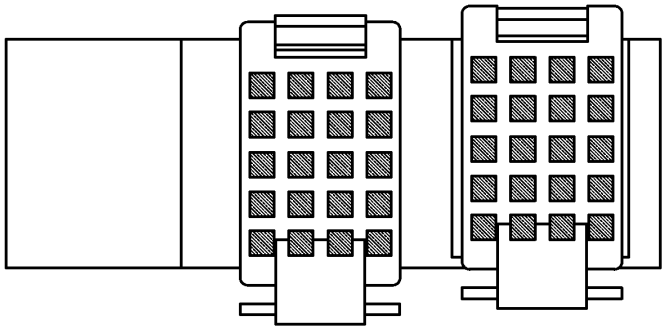


FIG. 9B

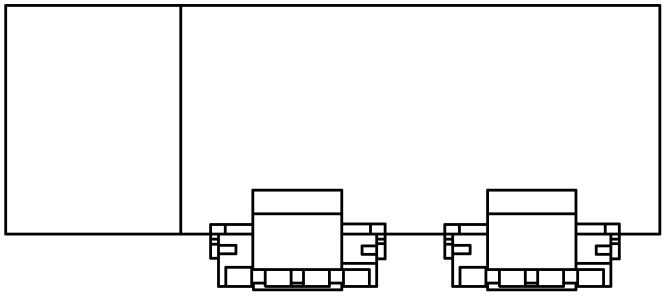


FIG. 9A