

Quantitative Fluorescence Assays Using a Self-Powered Paper-Based Microfluidic Device and a Camera-Equipped Cellular Phone

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Supporting Information

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Materials Used

Tape used was Ace Hardware brand plastic carpet tape (product # 50106). The paper was Whatman Chromatography Paper #1. Sodium nitrate, silver nitrate, aluminum chloride, silver sheet, aluminum sheet, β -D-galactosidase, fluorescein, and mono- and di-basic sodium phosphate were all purchased commercially and used without further purification. The filter used is a Kodak Wratten 2 filter No. 12, purchased from Edmond Optics (product # NT45-467). The LED was purchased from DigiKey (product # P14150CT-ND). The cell phone case is the Metallic Slider Case (steel) for iPhone 4S made by Incase Designs Corp.

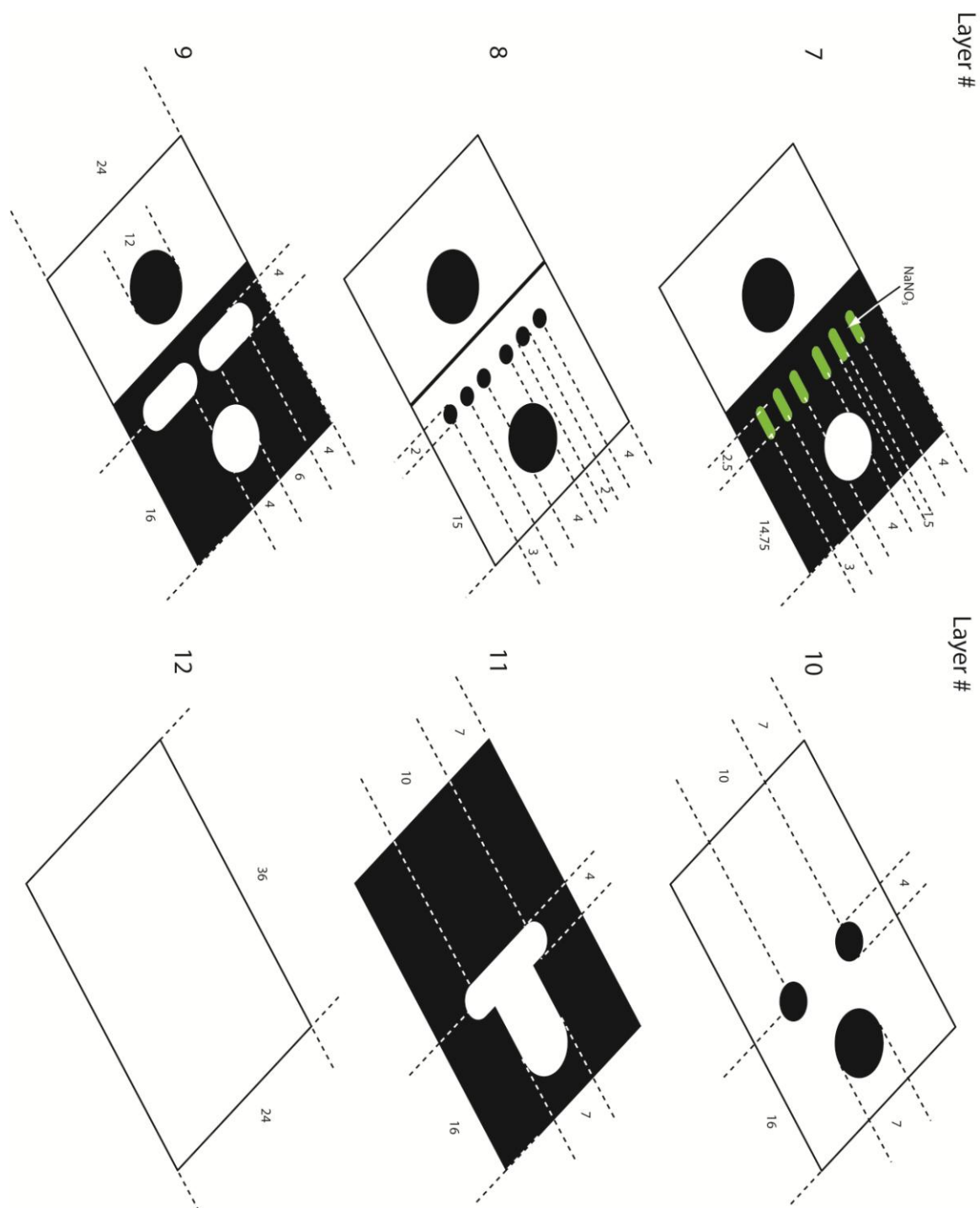


Figure S1 Continued. Layers of the assay device with dimensions labeled. The values are all listed in mm. The half of layers 5–9, which is constantly, tape, is the assay side of the device, and the other side (which alternates between paper and tape) is the battery side of the device.

Patterning paper and tape layers

Patterning the paper and tape layers has been reported previously by Noh et al.¹

Assembly of the device

The wax on the paper layers was melted for 105 s on a 150° C hot plate with a stack of papers on top of it. After cooling, the battery side of layer 7 had salt added to it, 0.75 μL of saturated NaNO_3 solution was added in two aliquots, drying under vacuum for 30 min after each addition. The battery side of layer 5 had 0.25 μL of the appropriate metal solution placed in each spot. The metal solutions used were 1 M AlCl_3 and 3 M AgNO_3 . The placement of the salts can be seen in Figure S1. After the addition of the salts, layer 5 was dried under vacuum for 30-60 min.

The protective layer on one side of layer 10 was removed, and layer 10 was added to layer 11, with the adhesive attaching them together. Layer 11 was then cut out from the surrounding paper, with scissors, using layer 10 as a guide. The protective layer on one side of layer 12 was removed and layer 12 was adhered to layer 11.

The battery side of layer 8 had one protective cover removed, and it was adhered to the battery side on layer 9. These were then cut out, with scissors, using layer 8 as a guide. The remaining protective sheet on layer 10 was then removed, the holes were filled with technicloth discs of the appropriate sizes, and layer 9 was attached.

The battery side on layer 6 then had one protective sheet removed and was attached to the battery side on layer 7 (already containing the salts). These are then cut out, with scissors, using layer 6 as a guide. The remaining protective sheet on layer 8 was then removed, technicloth discs of the appropriate sizes are placed in the holes, and layer 7 was attached.

The inner edge on the battery side of layer 5 was cut out, making sure that it is short enough to not overlap with the assay side of the layer. A protective sheet on layer 4 was then removed, and layer 4 was attached to layer 5. The assay sides of layers 5–9 were all stacked onto layer 4. Then the layer 4 stack was attached to the stacked layers for the bottom of the device, with technicloth discs placed in the holes in layer 6. A piece of black electrical tape was cut and placed at the bottom of the assay region, and the entire stack is placed under a 4.6 kg weight for at least 15 minutes (but not longer than 2 days) before continuing.

The 10 copies of layer 3 were then stacked together, and set aside. Layer 2 was attached to layer 1, and the pieces were then cut out, with scissors, and this was set aside. Clear plastic straws were cut into 2 mm tall cylinders using a razor, and these were set aside. The protective layer on layer 4 was removed and pieces of Al and Ag are placed in the line of holes further from the assay side of the device. Each piece of metal had a small amount of gallium placed on top, and a piece of Cu tape was placed down connecting all 6 pieces of metal. A thin strip of carpet tape was then cut and placed over the copper tape, and the protective layer on the carpet tape was removed.

The Ag pieces were then placed in the remaining Ag holes, a small amount of gallium was placed on each metal piece, and a strip of Cu tape was placed connecting all three pieces. This was repeated for the Al pieces in the Al holes. A thin piece of carpet tape is placed in between the two pieces of Cu tape, above where the LED will be placed, and the protective sheet is removed.

The stack of layer 3 was then attached to the top of layer 4. The LED was placed, with the ground on the Al side of the copper tape, and a small amount of gallium was used to attach the LED to the separate pieces of Cu tape. One piece of straw is placed in the assay region, directly in front of the LED. The battery input region hole is then filled with technicloth discs and covered with layers 1 and 2.

Characterization of the Battery

Measuring current and potential of the battery

To measure the current and potential of this battery, the device was built as described above, without including layer 3, the LED, or the piece of straw. Alligator clips were used to attached the pieces of copper tape to a multimeter, which was used to monitor either current or potential. The battery was run with 80 μ L deionized water.

Table S1. Data collected for the current and potential of the battery.

min Time	mA Trial 1	mA Trial 2	mA Trial 3	V Trial 4	V Trial 5
0	0	0	0	0	0
0.5	0	0	0	0.02	0.02
1	0	0	0	0.02	0.02
1.5	0	0	0	0.04	0.03
2	0	0	0	0.04	0.03
2.5	0	0	0	0.05	0.04
3	0	0	0	0.72	0.06
3.5	0	0	0	1.75	0.92
4	0	0.05	0	2.58	2.22
4.5	0	0.24	0.01	2.74	2.65
5	0	0.39	0.09	2.8	2.69
5.5	0.07	0.52	0.22	2.86	2.68
6	0.28	0.6	0.4	2.87	2.66
6.5	0.39	0.64	0.53	2.84	2.67
7	0.58	0.64	0.6	2.88	2.68
7.5	0.61	0.63	0.67	2.91	2.68
8	0.63	0.64	0.68	2.92	2.7
8.5	0.64	0.65	0.67	2.92	2.68
9	0.65	0.61	0.65	-	-
9.5	0.65	0.55	0.62	-	-
10	0.64	0.46	0.62	-	-
10.5	0.58	0.4	0.52	-	-
11	0.55	0.37	0.58	-	-
11.5	0.49	0.27	0.5	-	-
12	0.41	0.25	0.39	-	-
12.5	0.31	0.22	0.35	-	-
13	0.17	0.19	0.29	-	-
13.5	0.11	0.13	0.26	-	-
14	0.11	0.12	0.25	-	-
14.5	0.1	0.12	0.23	-	-
15	0.09	0.11	0.28	-	-
15.5	-	0.1	0.26	-	-
16	-	0.1	0.17	-	-

Table S1 Continued. Data collected for the current and potential of the battery.

min	mA	mA	mA	V	V
Time	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
16.5	-	0.1	0.1	-	-
17	-	0.09	0.12	-	-
17.5	-	-	0.1	-	-
18	-	-	0.18	-	-

Measuring LED output over time

The LED output over time was measured using the same fluorescence device described above, with a few small changes. Layers 1 and 2 were extended the whole length of the device, and a small hole (1.5 mm diameter) centered over the straw. A variable photoresistor had a small ring of tape placed on the edge of it, and it was centered and taped down onto the hole over the assay region. The battery was started with 80 μ L deionized water and the resistance was measured over time.

Table S2. Data collected for LED output over time.

min	k Ω	k Ω	k Ω	k Ω	k Ω
Time	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
0	2015	2015	2015	2015	2015
0.5	2015	2015	2015	2015	2015
1	2015	2015	2015	2015	2015
1.5	2015	2015	2015	2015	2015
2	2015	2015	2015	2015	2015
2.5	2015	2015	2015	2015	2015
3	2015	2015	2015	2015	2015
3.5	2015	1049	2015	2015	2015
4	2015	467	2015	2015	2015
4.5	2015	267	2015	2015	2015
5	2015	221	2015	1200	2015
5.5	2015	210	2015	569	2015
6	2015	204	2015	405	1760
6.5	2015	215	2015	285	878
7	2000	218	1580	240	512
7.5	2000	222	930	215	440
8	646	225	740	197	385
8.5	609	229	620	185	345
9	578	231	535	175	308
9.5	540	234	420	166	283
10	515	238	395	160	265
10.5	503	225	375	156	253
11	490	233	355	152	242

Table S2 Continued. Data collected for LED output over time.

min Time	kΩ Trial 1	kΩ Trial 2	kΩ Trial 3	kΩ Trial 4	kΩ Trial 5
11.5	475	238	335	150	233
12	452	238	320	147	225
12.5	434	240	309	145	222
13	420	243	298	143	217
13.5	412	261	290	142	213
14	407	249	278	141	210
14.5	399	250	270	141	208
15	392	271	262	141	205
15.5	383	271	258	142	203
16	373	255	254	143	202
16.5	368	254	250	143	201
17	366	258	245	144	199
17.5	365	283	243	144	198
18	364	270	240	144	199
18.5	362	269	238	145	200
19	361	273	235	146	200
19.5	360	276	234	147	200
20	361	282	233	148	202
20.5	359	286	233	150	202
21	360	290	233	151	203
21.5	363	295	233	152	203
22	367	299	233	153	203
22.5	368	304	235	154	203
23	371	316	236	154	204
23.5	378	330	237	154	203
24	378	341	240	154	206
24.5	382	360	243	155	207
25	387	379	245	156	207
25.5	393	-	247	158	209
26	400	413	248	159	211
26.5	408	433	252	160	212
27	417	450	257	161	214
27.5	429	450	260	163	217
28	440	457	266	165	218
28.5	454	477	270	166	221
29	466	500	277	169	224
29.5	480	536	281	171	227
30	489	555	290	173	230

Fluorescein calibration curve

Making buffer

1 L pH 7.6 sodium phosphate buffer was made by dissolving 1.79 g (13 mmol) monobasic sodium phosphate monohydrate and 12.35 g (87 mmol) dibasic sodium phosphate anhydrous in deionized water. The pH of this solution was found to be 7.61.

Making fluorescein solutions

11.6 mg (0.035 mmol) fluorescein free acid was dissolved in 500 mL of the buffer solution, giving a 69.8 μM stock solution. The stock solution was serially diluted with the buffer solution to give the tested concentrations: 5.93 μM , 5.00 μM , 4.00 μM , 3.00 μM , 2.00 μM , 1.00 μM , 0.50 μM and 0.25 μM fluorescein in sodium phosphate buffer.

Cell phone case design

The cell phone case (Figure S2) was designed to place the assay at the nearest focal point of the camera while blocking out all external light. The case was purchased commercially, and a black polyethylene tube (cut to 2") was glued onto the case over the cutout for the camera. Two caps were designed for used on the end of the tube. One contained a filter (Kodak Wratten 2 No. 12) to remove excess LED light from the picture (Figure S3); the second cap was used to keep the focal length the same for pictures with and without the filter.

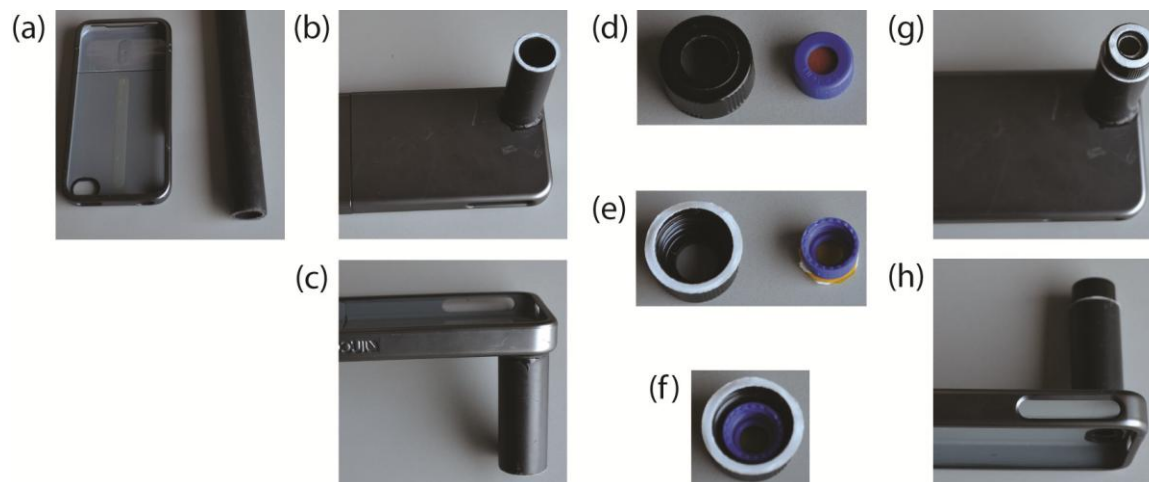


Figure S2. Picture of the cell phone case put together and of the various pieces, showing how it is put together. (a) The cell phone case and black tube as purchased. (b) the tube cut to 2" long and glued onto the cell phone case. The white end on the tube is a piece of tape. (c) The tube glued onto the cell phone case. (d) The two vial lids that are used to make a cap for the end of the tube. (e) Both vial lids with the septa removed. The black lid has a tape ring on the bottom and top, and the blue lid has a ring of tape on top holding on the yellow filter, and a second ring of tape on the other side of the filter. (f) The two lids stacked to form the cap. The blue lid is taped into the black lid. (g) The cap placed onto the cell phone case with the tube. A single protective layer from the tape remains between the cap and the tube, allowing the cap to be adhered for pictures, but removable to switch to the cap without the filter. The ring of tape on the top of the cap also contains a protective layer. This is used to adhere the cap to the fluorescence device to block out all light when taking a picture, and the protective layer allows for easy removal from the device. (h) a second angle of the device in (g).

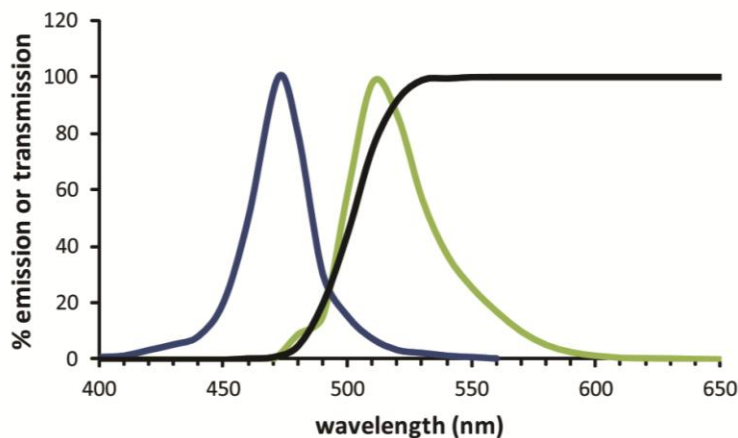


Figure S3. Graph showing the range of wavelengths for each component in the assay. This shows that the filter used is able to block out most of the background LED light, while allowing through most of the emission light. The blue data is the LED emission, the green data is the fluorescein emission, and the black line is the transmission through the filter.

Making the calibration curve with fluorescein solutions

To run an assay, 80 μL deionized water is spotted on the battery side of the device, and 70 μL of the sample is spotted in the assay region. The device was then connected to the case without a filter, and after 16-17 min a picture was taken, the case was then switched to containing a filter and a second picture was taken. This as repeated 3-4 times for each concentration of fluorescein solution tested.

Table S3. Data collected for the calibration curve of buffered fluorescein using different LEDs for every trial, without using a filter or the cell phone case. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (μM)	Trial 1	Trial 2	Trial 3	Trial 4
0	31	31	10	10
1	18	19	37	38
1.5	47	59	30	30
2.5	61	55	23	24
5	80	87	117	120
7.5	53	48	151	58
10	88	92	51	93

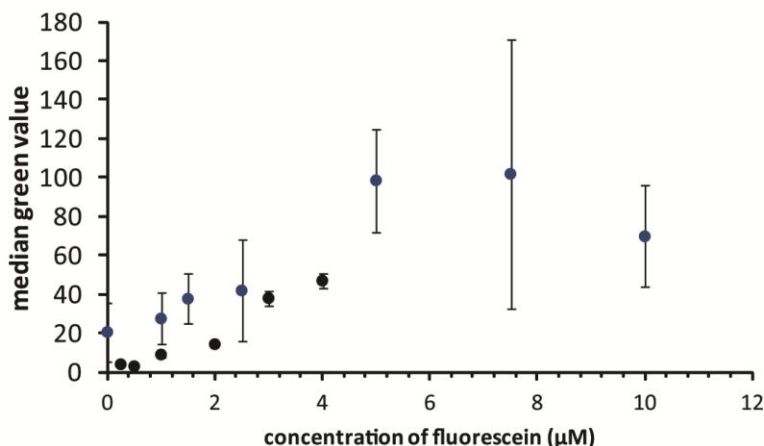


Figure S4. Graph showing the relative improvement in reproducibility for measuring fluorescein from the implementation of the various features of the device. The blue data is from devices imaged with a cell phone camera only, with no case, filter, or alignment circles used. The black data is from devices imaged using a cell phone camera as described above.

Table S4. Data collected for the calibration curve of buffered fluorescein using different LEDs for every trial, without using a filter. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (μM)	Trial 1	Trial 2	Trial 3	Trial 4
0	17	13	-	-
0.25	12	14	20	-
0.5	28	37	14	-
1	72	86	45	49
2	93	19	29	-
3	57	70	33	-
4	121	-	65	-
5	82	150	170	104

Table S5. Data collected for the calibration curve of buffered fluorescein using different LEDs for every trial, using a filter. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (μM)	Trial 1	Trial 2	Trial 3	Trial 4
0	5	5	-	-
0.25	6	5	7	-
0.5	8.5	10	5	-
1	25.5	30	10	16
2	33	16	10	-
3	27	31	17	-
4	54	-	29	-
5	51	66	84	50

Table S6. Data collected for the calibration curve of buffered fluorescein using the same LEDs for every trial, without using a filter. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (μM)	Trial 1	Trial 2	Trial 3
0.25	3	-	-
0.5	4	-	-
1	22	21	25
2	25	41	35
3	71	80	52
4	77	96	73
5	80	76	100
5.93	52	96	75

Table S7. Data collected for the calibration curve of buffered fluorescein using different LEDs for every trial, using a filter. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (μM)	Trial 1	Trial 2	Trial 3
0.25	3	-	-
0.5	4	-	-
1	8	10	9
2	13	16	14
3	37	42	35
4	43	51	47
5	35	46	37
5.93	27	54	42

Assay for β -D-galactosidase

Synthesis of the assay reagent

General Experimental Procedures

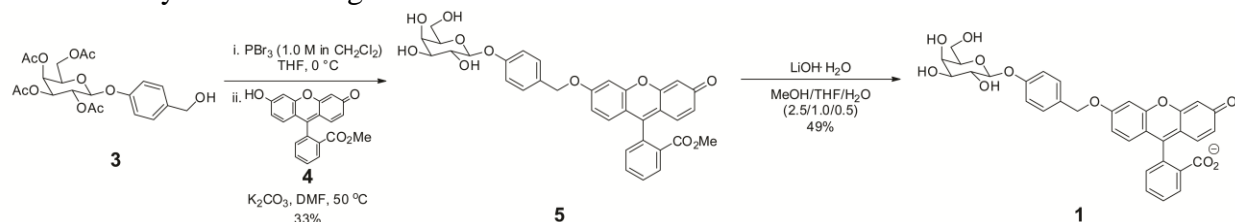
All reactions that required anhydrous conditions were performed in flame-dried glassware under a positive pressure of argon. Air- and moisture-sensitive liquids were transferred by syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation (25–40 mmHg) at ambient temperature, unless otherwise noted. Thin layer chromatography was carried out on Dynamic Adsorbants silica gel TLC (20 \AA –20 cm w/h, F-254, 250 μm).

Instrumentation

Proton nuclear magnetic resonance (^1H -NMR) spectra and carbon nuclear magnetic resonance spectra (^{13}C -NMR) were recorded using a Bruker CDPX-300 (300 MHz) or AV-360 (360 MHz) at 25 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to methanol (CD_3OD , 3.31 ppm). Data are represented as follows: chemical shift,

multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, br s = broad singlet, dd = doublet of doublet), integration, and coupling constant (*J*) in Hertz. Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to methanol (CD₃OD, 49.0 ppm).

Scheme 1. Synthesis of reagent 1.



Methyl 2-(3-oxo-6-(4-((2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)benzyloxy)-3*H*-xanthen-9-yl)benzoate (5):

Compound **3**² (0.20 g, 0.44 mmol, 1.0 equiv) was dissolved in dry tetrahydrofuran (4.4 mL) and the solution was cooled to 0 °C. Phosphorous tribromide (1.0 M solution in dichloromethane, 180 μ L, 0.18 mmol, 0.4 equiv) was added dropwise, and the solution was stirred at 0 °C for 20 min. The reaction was diluted with dichloromethane (10 mL) and quenched with saturated sodium bicarbonate (20 mL). The organic layer was collected, and the aqueous layer was extracted with dichloromethane (3 \times 20 mL). The combined organic layers was dried over sodium sulfate, filtered, and concentrated by rotary evaporation, yielding a white solid. The white solid was used without further purification.

The white solid (0.13 g, 0.26 mmol, 1.0 equiv), compound **4**³ (0.09 g, 0.26 mmol, 1.0 equiv), and potassium carbonate (0.04 g, 0.29 mmol, 1.1 equiv) were dissolved in dry dimethylformamide (3 mL). The solution was heated at 50 °C for 19 h. The reaction mixture was cooled to room temperature and the solvent was removed by rotary evaporation. The residue was purified by preparative HPLC to afford compound **5** as an orange solid (52 mg, 85 μ mol, 33%). IR (cm⁻¹): 3328.9, 2923.2, 1723.2, 1589.9; ¹H-NMR (300 MHz, CD₃OD): δ 8.31 (dd, 1 H, *J* = 2.07, 9.19), 7.87 – 7.75 (m, 2 H), 7.44 – 7.41 (m, 1 H), 7.42 (d, 2 H, *J* = 10.44), 7.29 (d, 1 H, *J* = 2.71), 7.16 (d, 2 H, *J* = 10.48), 7.05 – 6.95 (m, 3 H), 6.59 (dd, 1 H, *J* = 2.45, 11.53), 6.49 (d, 1 H, *J* = 2.46), 5.21 (s, 2 H), 3.91 (d, 1 H, *J* = 3.5), 3.83 – 3.67 (m, 5 H), 3.61 (s, 3 H), 3.61 (dd, 1 H, *J* = 6.46, 14.12); ¹³C-NMR (360 MHz, CD₃OD): δ 187.2, 167.0, 166.0, 161.5, 159.3, 156.3, 135.5, 134.1, 132.6, 132.2, 131.8, 131.5, 131.3, 131.0, 130.7, 130.5, 129.3, 118.1, 117.9, 116.3, 105.5, 102.9, 102.5, 77.0, 74.9, 72.3, 71.8, 70.2, 62.4, 52.9; MS (TOF MS AP+, *m/z*): 615.1 (*M* + 1); HRMS (TOF MS ES+) Calculated for C₃₄H₃₁O₁₁ (*M* + 1): 615.1866; Found 615.1862.

2-(3-Oxo-6-(4-((2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)benzyloxy)-3*H*-xanthen-9-yl)benzoic acid (1):

Compound **5** was dissolved in dry methanol (950 μ L) and tetrahydrofuran (380 μ L), and the solution was stirred at room temperature. To the stirring solution was added a solution of LiOH·H₂O in deionized water (6 mg, 0.14 mmol, 180 μ L). The reaction mixture was stirred at room temperature for 4 h and an additional portion of LiOH·H₂O in deionized water (6 mg, 0.14 mmol, 180 μ L) was added. After 17 h, the mixture was diluted with water and carefully

acidified using Dowex® C-211 H⁺ exchange resin (final pH = 5). The resin was removed by filtration, and the filtrate was concentrated under reduced pressure, yielding a yellow residue. The residue was purified by preparative HPLC to afford compound **1** as a yellow solid (21 mg, 35 μmol, 49%). IR (cm⁻¹): 3343.5, 2924.2, 1737.4; ¹H-NMR (360 MHz, CD₃OD): δ 8.03 (d, 1 H, *J* = 6.88), 7.75 – 7.66 (m, 2 H), 7.39 (d, 2 H, *J* = 8.68), 7.20 (d, 2 H, *J* = 6.81), 7.14 (d, 2 H, *J* = 8.72), 6.95 (s, 1 H), 6.75 (s, 2 H), 6.69 – 6.65 (m, 2 H), 6.55 (dd, 1 H, *J* = 2.38, 8.86), 5.09 (s, 2 H), 3.91 (d, 1 H, *J* = 3.21), 3.82 – 3.67 (m, 5 H), 3.60 (dd, 1 H, *J* = 3.39, 9.76); ¹³C-NMR (360 MHz, CD₃OD): δ 171.9, 162.7, 159.1, 155.4, 154.3, 152.8, 135.5, 131.7, 131.0, 130.7, 130.3, 130.2, 126.7, 126.2, 117.8, 113.9, 113.4, 112.4, 103.8, 102.9, 77.0, 74.8, 72.3, 71.1, 70.2, 62.4; MS (TOF MS AP+, *m/z*): 601.1 (*M* + 1); MS (TOF MS AP-, *m/z*): 599.1 (*M* – 1); HRMS (TOF MS ES+) Calculated for C₃₃H₂₉O₁₁ (*M* + 1): 601.1710; Found 601.1709.

Running an assay

A stock solution of **1** was made in phosphate buffer (200 mM, pH 7.6) and used for all β-D-galactosidase assays. Added to 900 μL of the stock solution was 100 μL of β-D-galactosidase in 200 mM phosphate buffer, pH 7.6. The solution was mixed for 30 minutes before 75 μL of the sample was added to the sample holder of the device. After 15 minutes of mixing, 90 μL of deionized water was added to the battery portion of the device to turn on the LED. After the sample was added to the device, the device was connected to the case, containing the filter, and the sample was imaged using an iPhone 4S. The images were analyzed using Adobe Photoshop, measuring the median green value for each sample using the histogram function.

Table S8. Data for the calibration curve for β-D-galactosidase. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

Concentration (nM)	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6
0	40	39	38	38	34	34
1	48	49	52	50	53	53
2	69	71	62	63	51	51
3	73	75	73	75	70	72
4	82	85	75	78	78	82
5	94	97	96	98	88	91
6	92	95	95	98	90	93
8	98	101	98	101	98	102
10	106	109	105	109	103	108

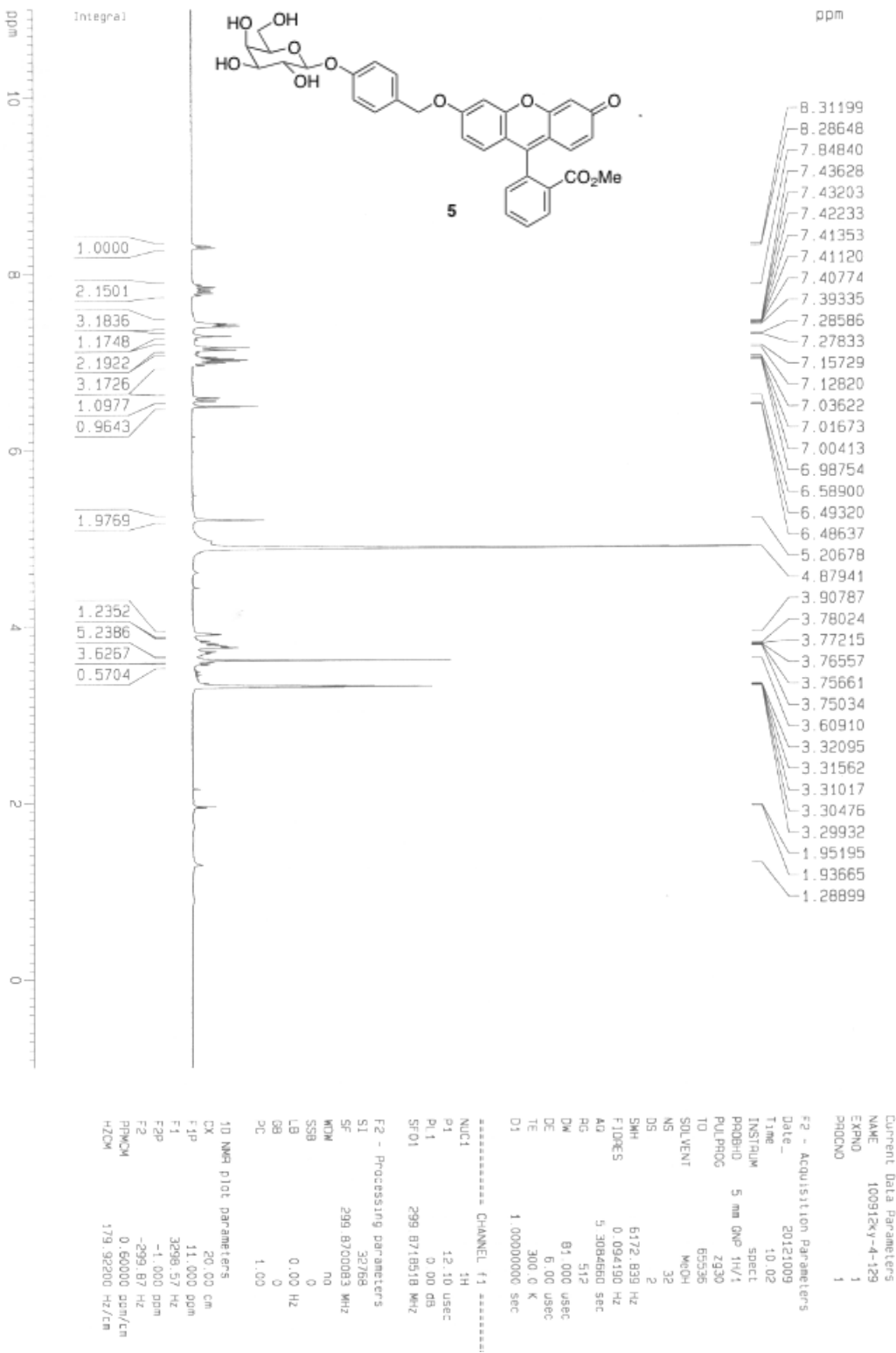
Table S8 Continued. Data for the calibration curve for β -D-galactosidase. All collected values are median value using the green channel of the histogram function in Adobe Photoshop.

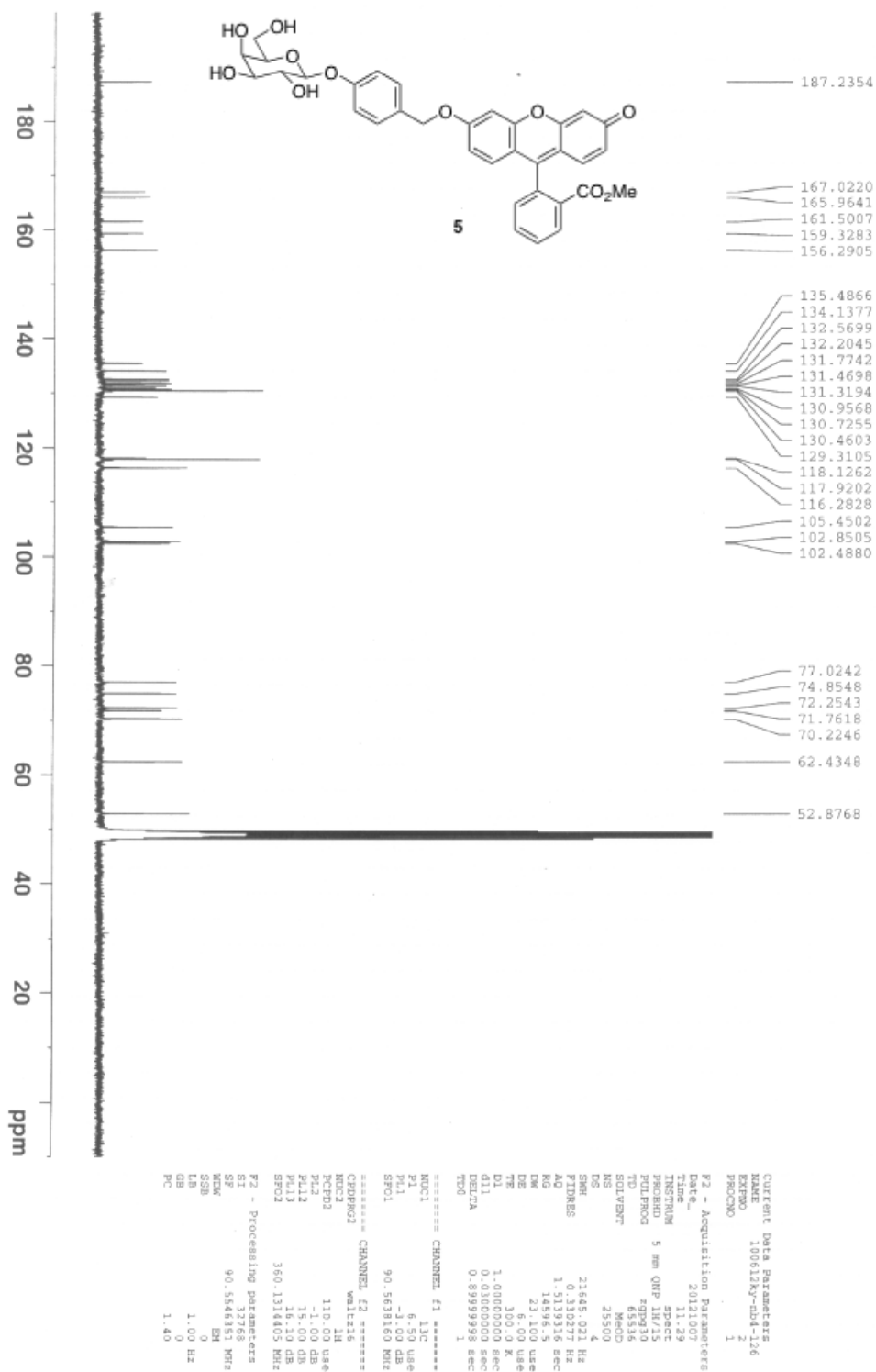
12	115	120	115	119	106	110
14	110	117	109	115	115	119
15	113	116	106	109	108	111
16	108	113	109	114	114	119
18	110	114	112	115	109	114
20	111	117	112	119	108	111

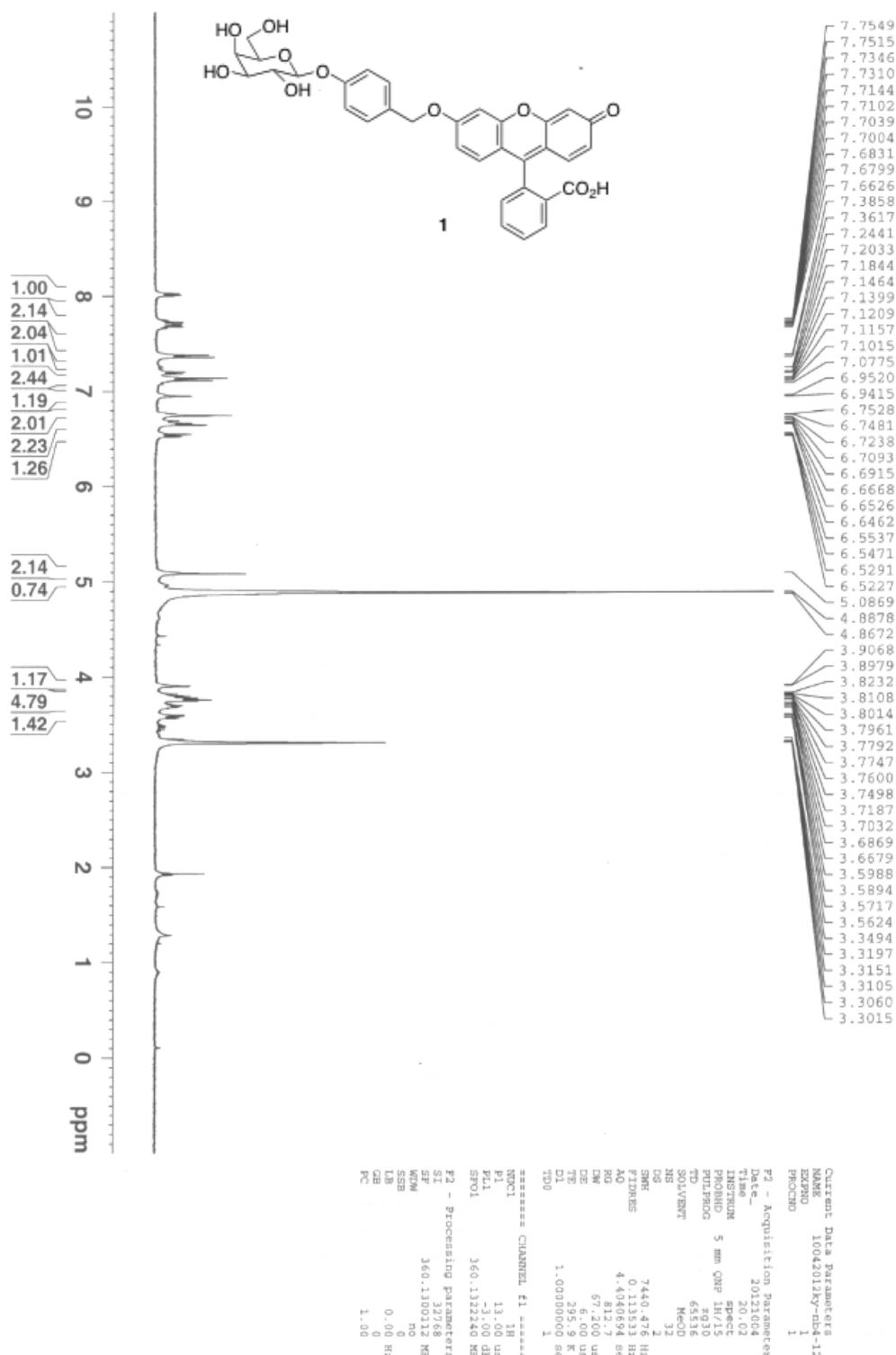
Running multiple assays in the same device

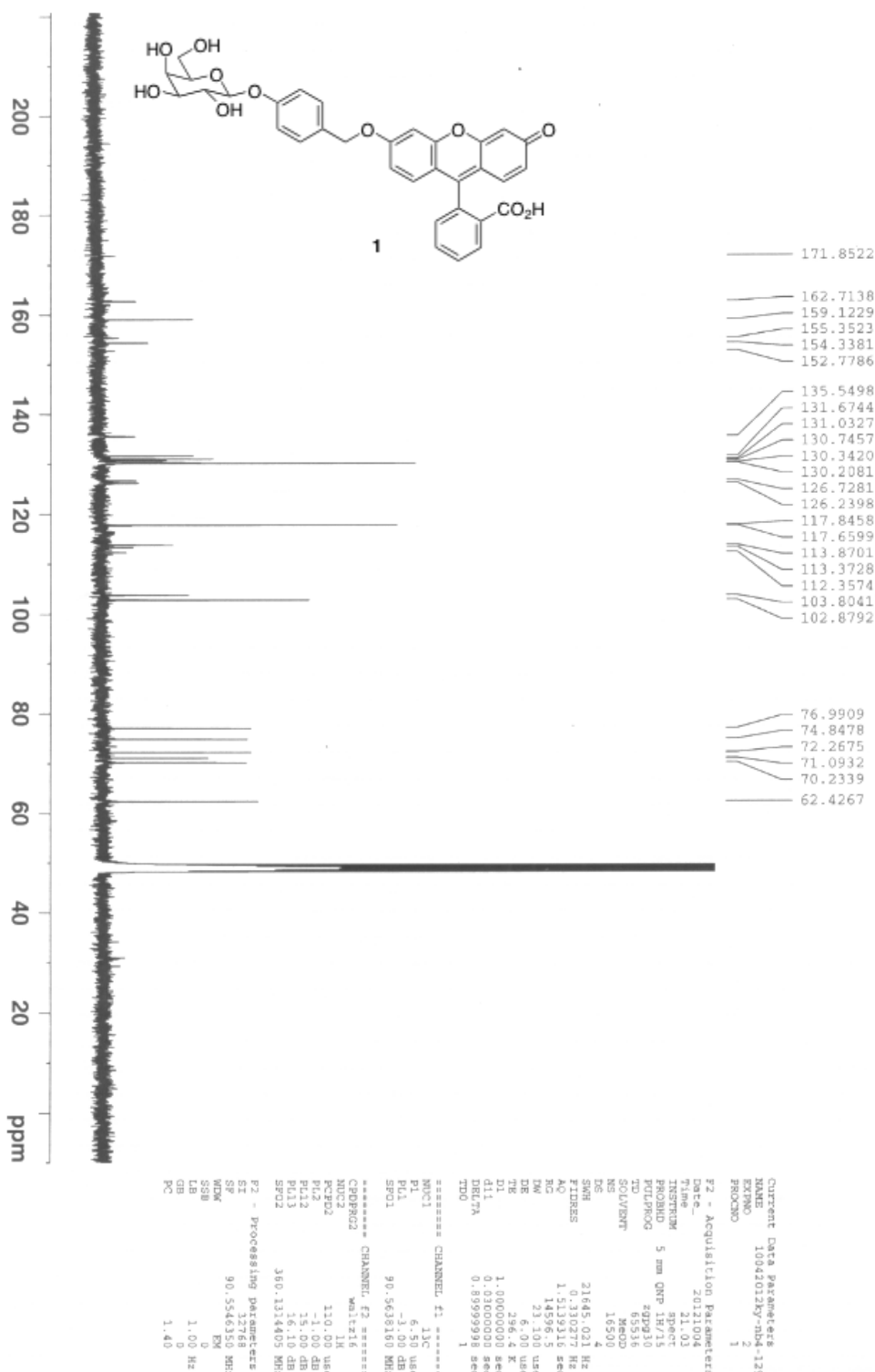
To run multiple assays in the same microfluidic device, the first assay solution is pipetted into the assay region, and pictures are taken, then the assay solution is removed. The assay region is rinsed with the next assay solution three times (by pipetting in and out the new solution), then the next assay solution is pipetted into the assay region and pictures are taken. This process is continued as long as the battery time is between 15 and 22 minutes.

Copies of NMR spectra









References

1. H. Noh, S. T. Phillips, *Anal. Chem.*, 2010, **82**, 8071.
2. A. K. Ghosh, S. Khan, D. Farquhar. *Chem. Commun.*, 1999, **24**, 2527.
3. M. Adamczyk, J. Grote, J. A. Moore. *Bioconjugate Chem.*, 1999, **10**, 544.