Electronic Supplementary Material (ESI) for Analyst.

Supporting information

Imaging Mass Spectrometry of Mouse Brain by Tapping-mode Scanning Probe Electrospray Ionization

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Materials and methods

SPESI ionization source

A fused silica capillary (Uncoated SilicaTip Emitters, inner diameter: 20 μm, outer diameter: 360 μm, tip inner diameter: 10 μm) New objective, USA) was used as a probe to supply solvent. A nano flow pump (Prominence nano, Shimadzu Corp., Japan) was connected to a probe with a PEEK union (Upchurch Scientific, USA). Bias voltage was applied to the solvents through a metal union (Upchurch Scientific) to generate electrospray ionization. A probe was vibrated with external piezo actuator (P-840.10, PI, Germany). A Function generator and voltage amplifier (WF 1946 and BA4825, NF Corp. Japan) was used to drive actuator. The solvent mixture (50:50 v/v DMF/EtOH) was supplied by nano-flow pump. The flow rate of solvent was 2 nl/min. The bias voltage both of the solvent and the ion extraction tube were -7.5 kV and -30 V, respectively. The vibration frequency of the piezo actuator was 278 Hz. The ion extraction tube (material: SUS, inner diameter: 3.1 mm, outer diameter: 1.7 mm) was heated at 300 degree C at the middle of the tube. The ion extraction tube was evacuated with the diaphragm vacuum pump (DA-40S, pumping speed: 40 L/min, ULVAC, Japan). The oscillating probe was observed with high-speed camera (VW-5000; Keyence, Osaka, Japan).

Data acquisition and analysis

A quadrupole time-of-flight (TOF) mass spectrometer (QSTAR XL, Applied Biosystems) was connected to external time to digital converter (TC890, Agilent Acqiris). Both of the data acquisition and the scanning of sample were controlled by PXI controller (PXIe-8133, NI, USA) and originally developed software (LabVIEW, NI, USA). Negative ion mode measurements at m/z 100-2000 (6.991 kHz of pulser frequency) were employed. The data acquisition at each XY position was executed to obtain 10,000 events of TOF measurements. The data acquisition time was about 1.5 sec. at each XY position. As the time interval between the sampling (formation of liquid bridge) and ionization steps are estimated to be about 1.8 msec, the number of sampling/ionization events for each XY position was estimated to be about 795.

Sample was XY moved at 100 μm pitch with auto-stage (5 Phase Stepping Motors, Sigma, Japan). The total pixels were 11096 (146 by 76 pixels). The construction of ion images was also processed by originally developed software (LabVIEW, NI, USA). Multiple ion images are constructed by calculating the signal intensity from 100 to 1,300 m/z at the 0.05 m/z step. The signal intensity at the specific m/z was normalized to construct 8-bit gray scale bitmap file. The color overlaid images were created by Image J

(NIH, USA). 248 ion images are selected from the tissue for a classification of multiple ion peaks as shown in Figure 3. The mass to charge ratio was calibrated with the mass spectrum of standard sample (YOKUDELNA, Jeol, Japan). The ion peaks are tentatively assigned from the database of lipidomics gateway (http://www.lipidmaps.org), METLIN: metabolite search (http://metlin.scripps.edu/), and previous reports. Both of the mass spectrometer, data acquisition system and the software for data analysis were newly developed.

Sample preparation

A brain of nude mouse (balb/cAcJ nu/nu) was rececte and frozen in liquid nitrogen immediately. Fresh-frozen tissues were cryo-sectioned by cryomicrotome (CRYO3; Sakura Finetek, Tokyo, Japan). The temperature of the sample was kept at –20 degree C, and the thickness of the tissue section was about 20 μm. The coronal section was mounted on a glass substrate (S7213; Matsunami Glass, Tokyo, Japan). Optimal cutting temperature (O.C.T.) compound (Tissue-Tek; Sakura Finetek, Torrance, CA, USA) was used to fix the sample onto the sample holder of the cryomicrotome. The sample was kept at -80 degree C until measurement. Before the experiment, sample was dried under atmosphere and used without further treatment.

Captions

Figure S1

Optical images of mouse brain section after the measurement with SPESI. The images both of cerebral cortex (Cbc) and corpus callosum (cc) indicate the traces that would be correspond to the area in which sampling/ionization occurred. The average length of 8 traces in Cbc and cc was 53 μm and 60 μm and 61 μm and 51 μm in X and Y directions, respectively. Note that no holes in the brain section were formed due to the probe impacts to sample surface. Scale bar: 100 μm.

Figure S2

The Mass spectrum of mouse coronal section at *m/z* 100 to 1000. Two kinds of mass spectrum of area 3 and 4 are shown. The peak position is also noted in the figure.

Figure S3

The catalog of ion images that were used for image grouping in Figure 3. The m/z value is noted in each image.

Figure S4

The mass spectrum of mouse coronal section at *m/z* 800 to 950. Each peak position of tentatively assigned sulfatides is noted in the figure. The 14 ion images of sulfatides are also shown on the right. The *m/z* for each images are noted inside image.

Figure S5

The mass spectra of all areas at *m/z* 720 to 764. Peaks tentatively assigned to gangliosides are noted in the image. The 6 ion images of are shown on the lower part. The m/z value is noted in each image.

Table S1

Tentative Assignments of major ion peaks of gangliosides from a mouse coronal brain section observed by using t-SPESI. All assignments are tentative and based on comparison against data from the METLIN Metabolite Database (http://metlin.scripps.edu/).

Figure S1

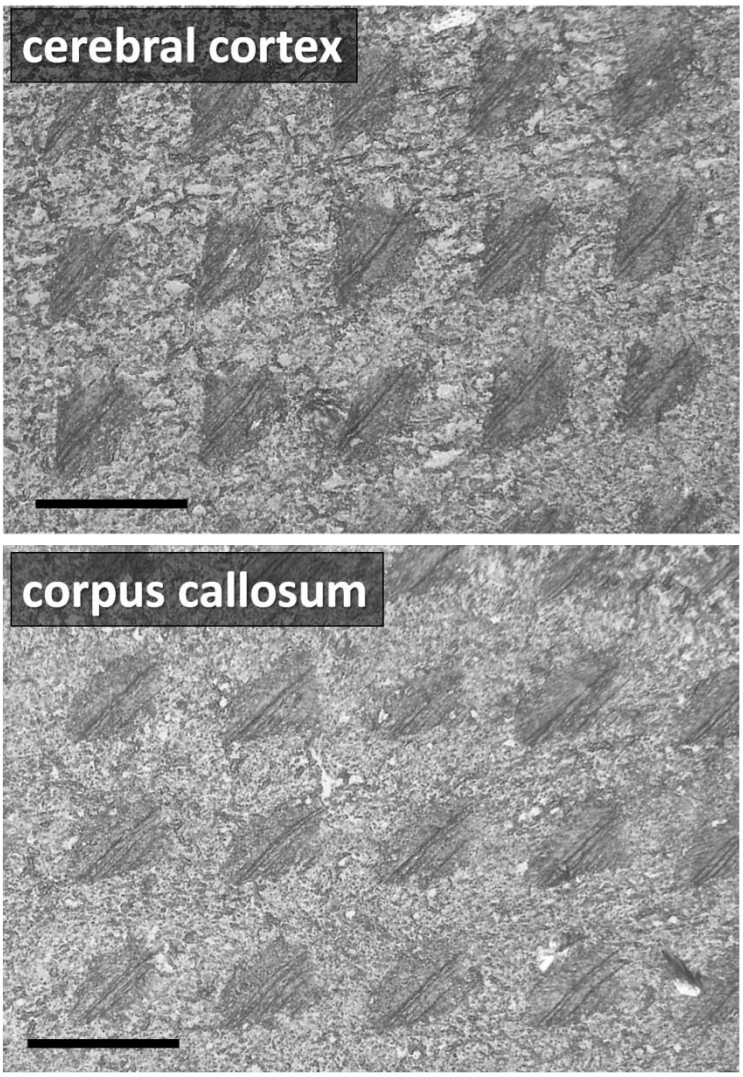
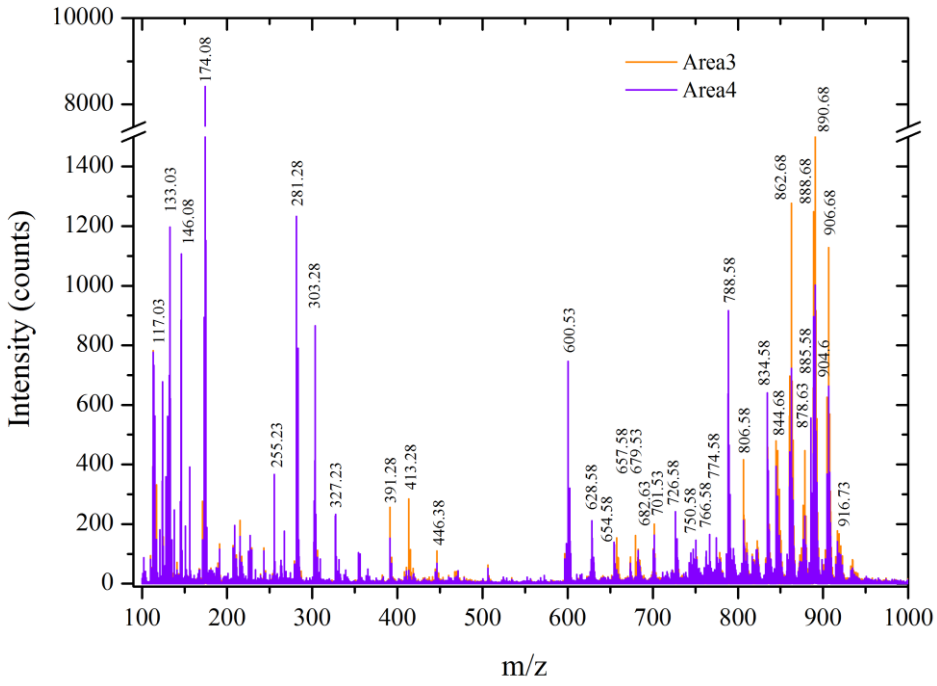


Figure S2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Area4
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m/z
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1200
1000
800
600
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300
400
500
600
700
800
900
1000
117.03
133.03
146.08
174.08
255.23
281.28
303.28
327.23
391.28
413.28
446.38
600.53
628.58
654.58
657.58
679.53
682.63
701.53
726.58
750.58
766.58
774.58
788.58
806.58
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890.68
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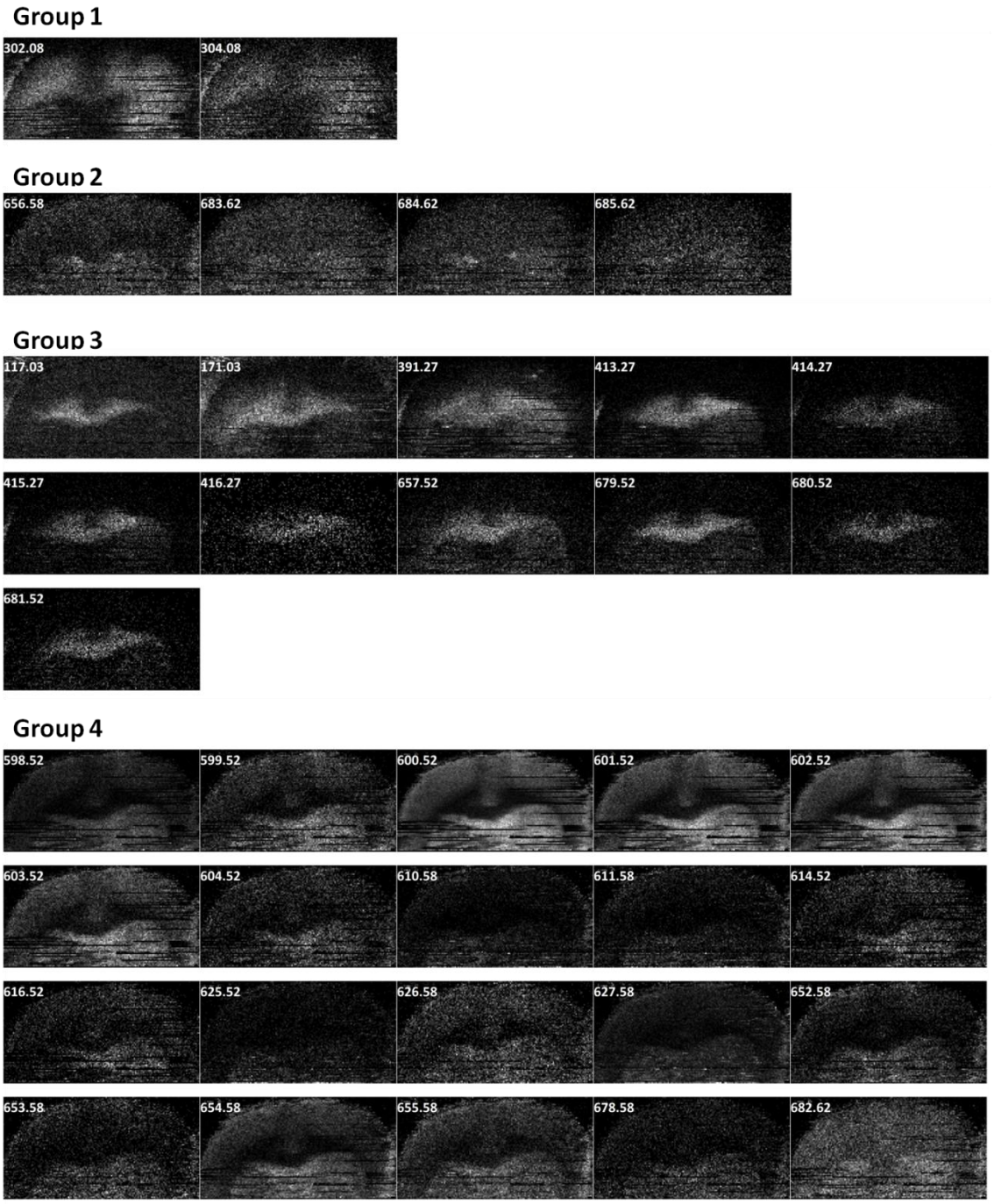
906.68
916.73

Figure S3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171.03
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414.27
415.27
416.27
657.52
679.52
680.52
681.52
Group 4
598.52
599.52
600.52
601.52
602.52
603.52
604.52
610.58
611.58
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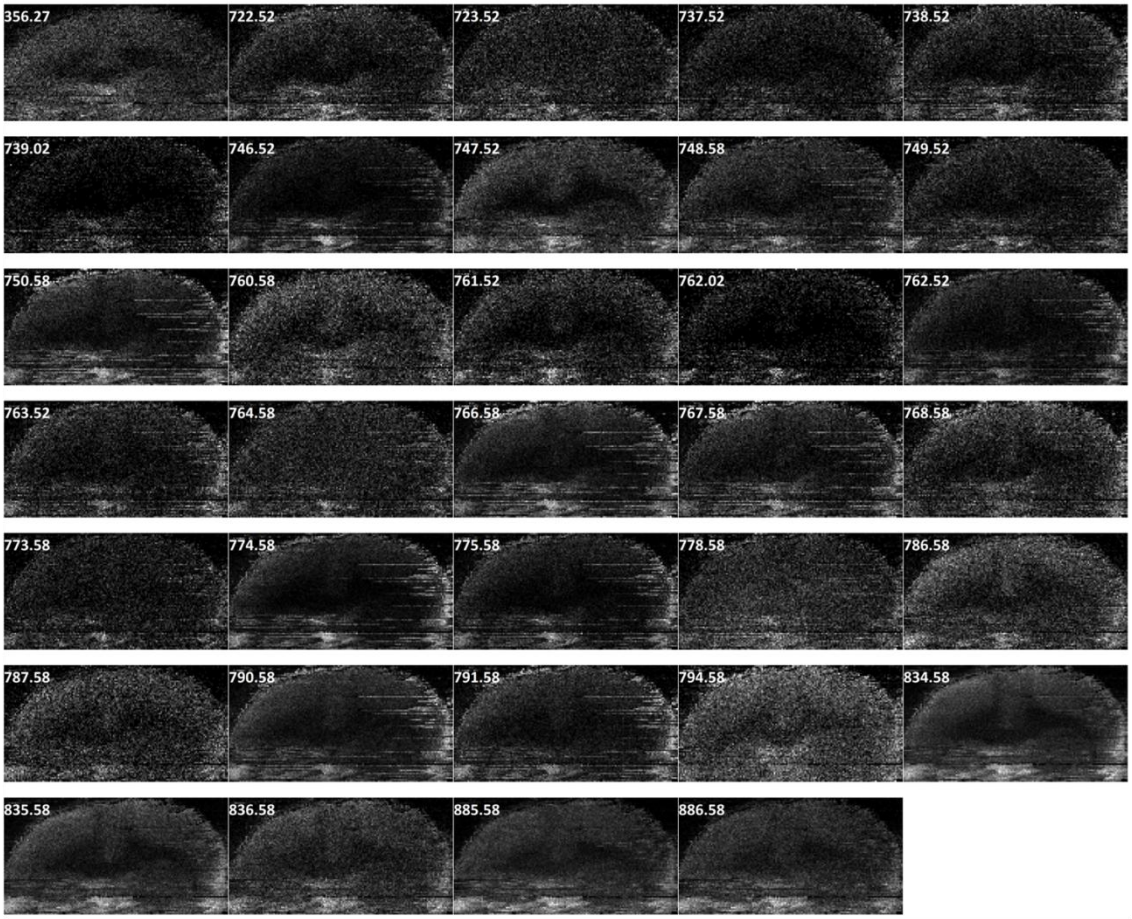
678.58
682.62

Group 5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761.52
762.02
762.52
763.52
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766.58
767.58
768.58
773.58
774.58
775.58
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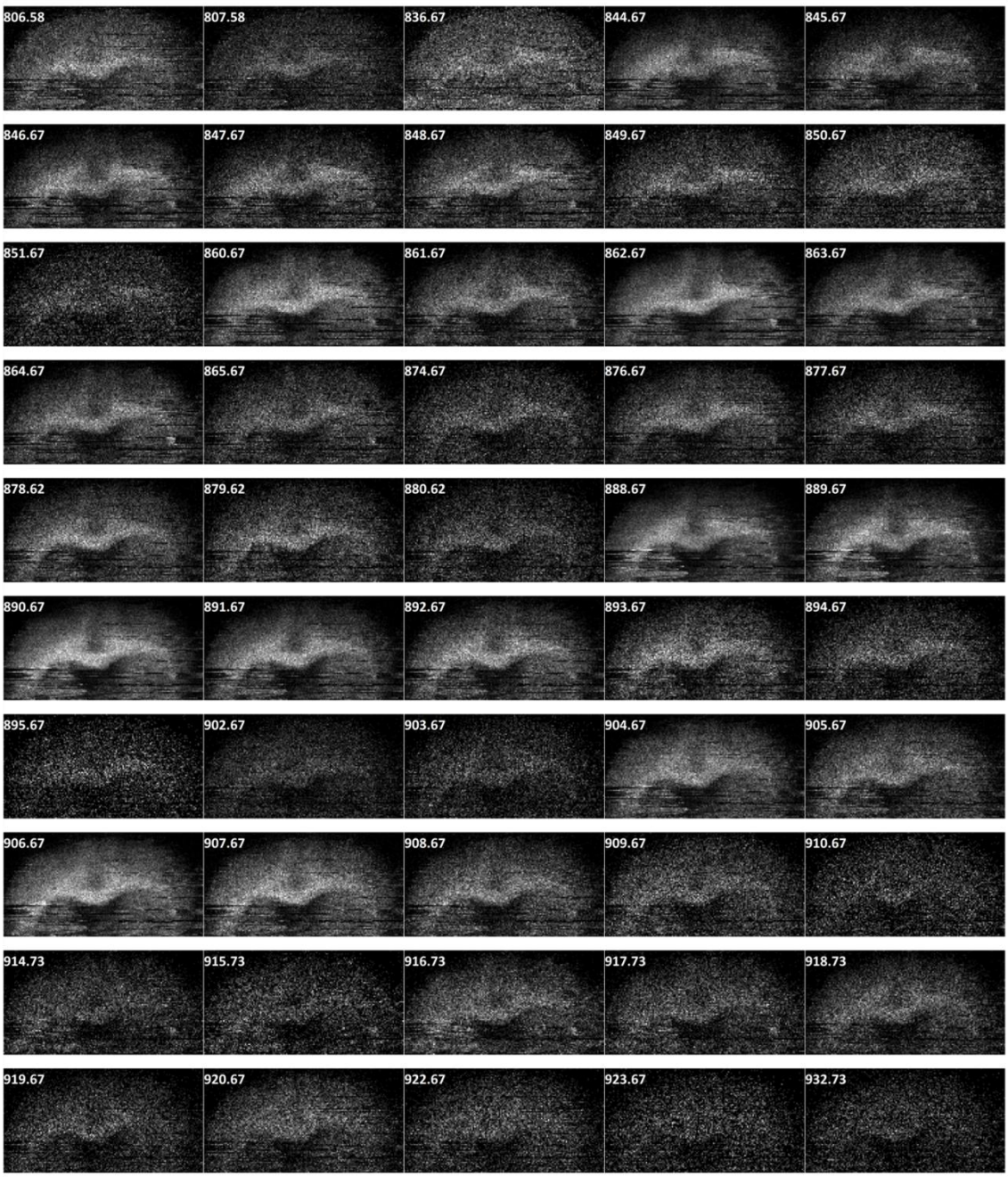
885.58
886.58

Group 6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862.67
863.67
864.67
865.67
874.67
876.67
877.67
878.62
879.62
880.62
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889.67
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891.67
892.67
893.67
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902.67
903.67
904.67
905.67
906.67
907.67
908.67
909.67
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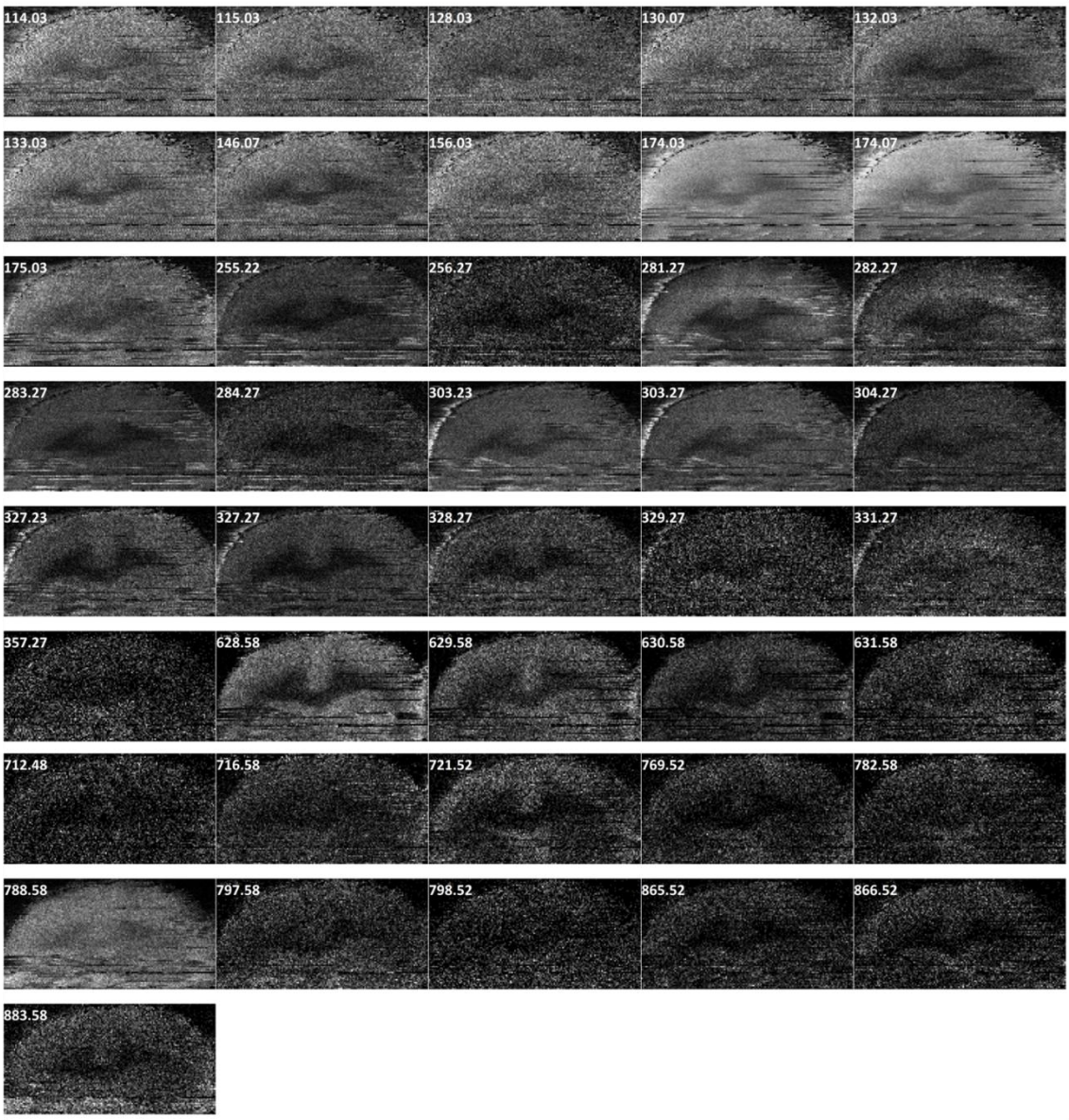
920.67
922.67
923.67
932.73

Group 7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282.27
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303.27
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328.27
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331.27
357.27
628.58
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630.58
631.58
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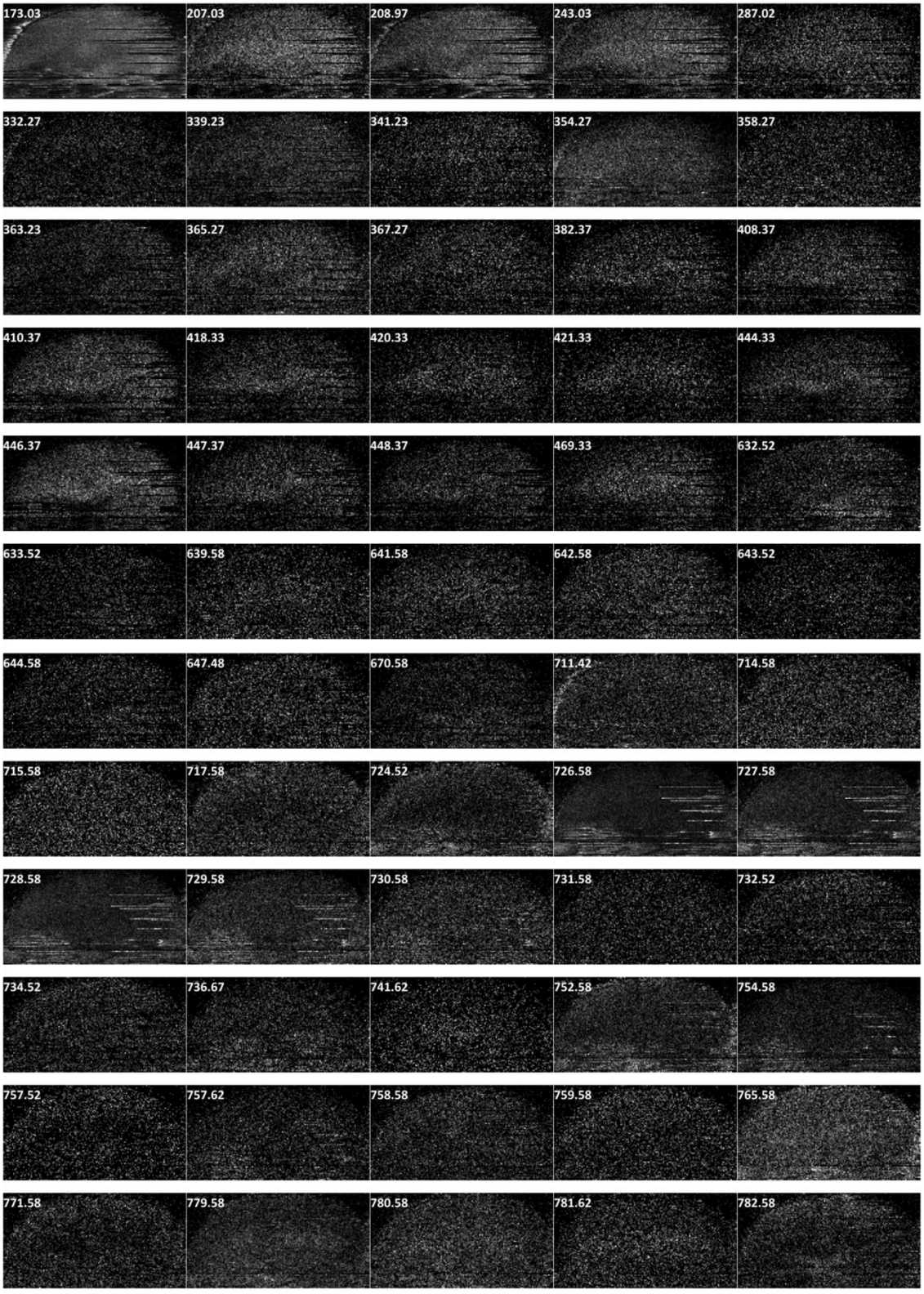
Group 8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Group 8

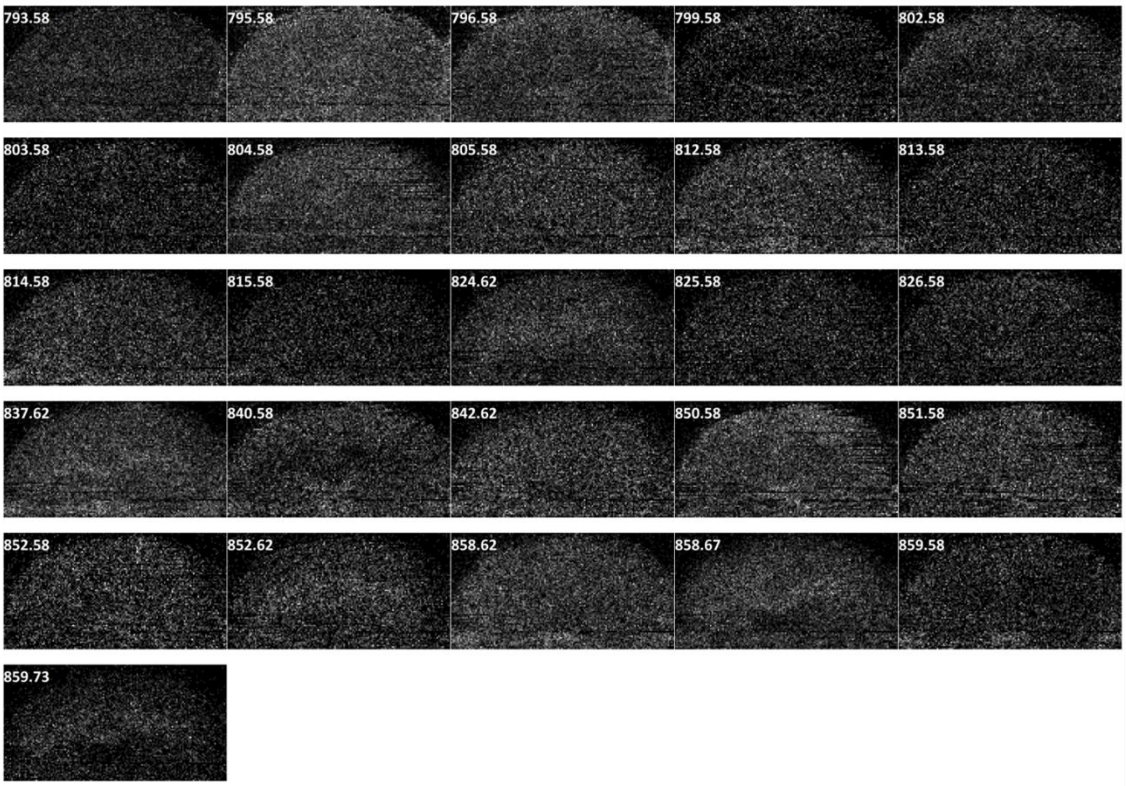
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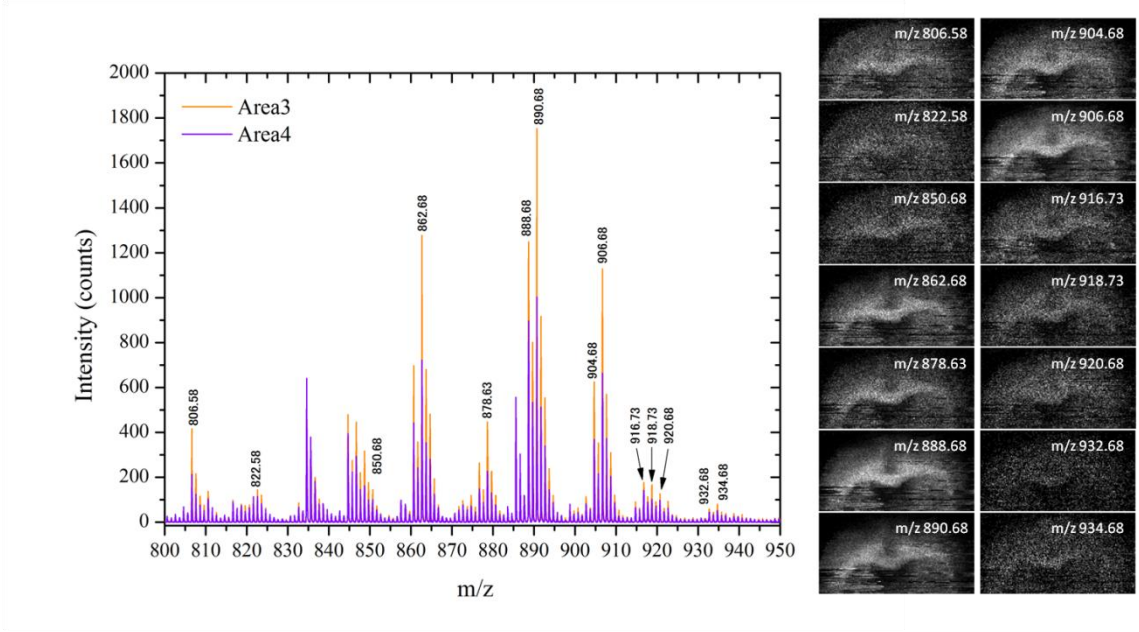
Figure S4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Area4
Intensity (counts)
m/z
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822.58
850.68
862.68
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904.68
906.68
916.73
918.73
920.68
932.68
934.68
m/z 806.58
m/z 904.68
m/z 822.58
m/z 906.68
m/z 850.68
m/z 916.73
m/z 862.68
m/z 918.73
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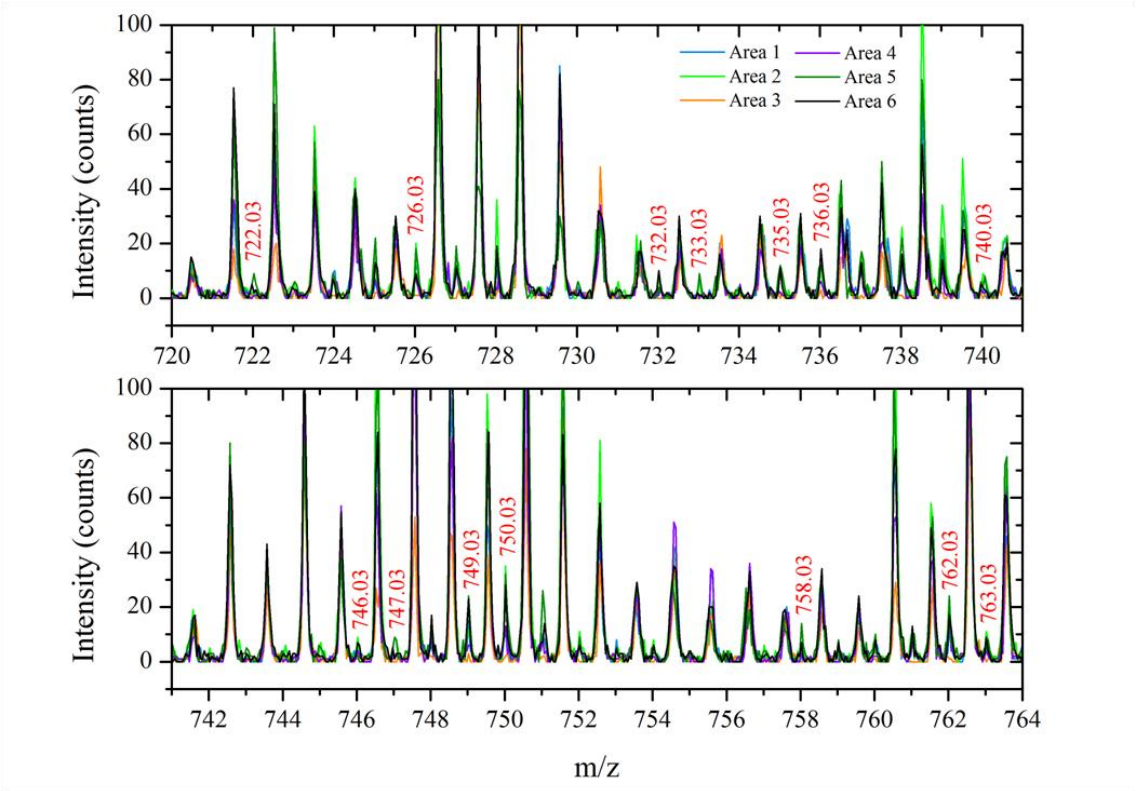
m/z 934.68

Figure S5


Area 1
Area 2
Area 3
Area 4
Area 5
Area 6
Intensity (counts)
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726.03
732.03
733.03
735.03
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750.03
758.03
762.03
763.03
m/z

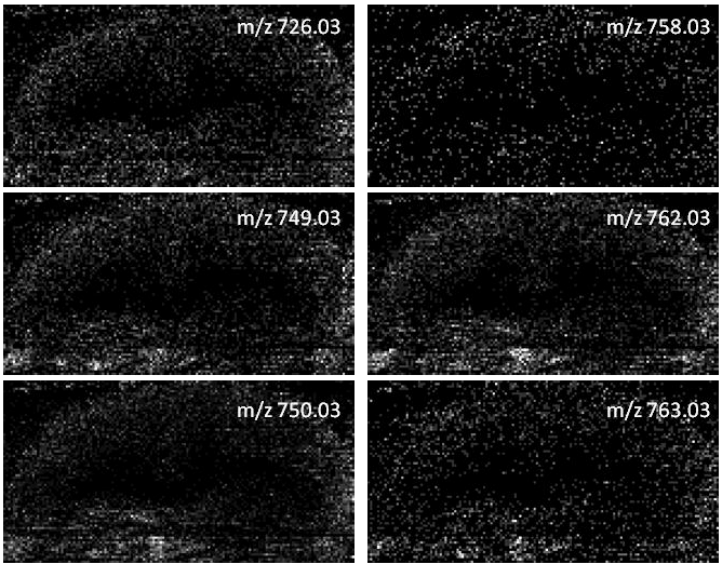
m/z 726.03
m/z 758.03
m/z 749.03
m/z 762.03
m/z 750.03
m/z 763.03

Table S1

Measured Mass	Ion Type	Exact Mass	Error ppm	Molecular Species	Formula
722.03	$[M-2H]^{2-}$	1445.824225	173	Ganglioside GD3 (d18:0/16:0)	C68H123N3O29
726.03	$[M-3H]^{3-}$	2181.119189	3	Ganglioside GT1b (d18:1/22:1(13Z))	C100H172N4O47
				Ganglioside GT1c (d18:1/22:1(13Z))	C100H172N4O47
732.03	$[M-2H]^{2-}$	1465.902035	117	Ganglioside GM2 (d18:1/24:1(15Z))	C73H131N3O26
	$[M-3H]^{3-}$	2199.166139	24	Ganglioside GT1b (d18:0/23:0)	C101H178N4O47
				Ganglioside GT1c (d18:0/23:0)	C101H178N4O47
733.03	$[M-2H]^{2-}$	1467.917725	106	Ganglioside GM2 (d18:1/24:0)	C73H133N3O26
735.03	$[M-2H]^{2-}$	1471.839875	159	Ganglioside GD3 (d18:1/18:0)	C70H125N3O29
736.03	$[M-2H]^{2-}$	1473.855525	148	Ganglioside GD3 (d18:0/18:0)	C70H127N3O29
	$[M-3H]^{3-}$	2211.166139	24	Ganglioside GT1b (d18:1/24:0)	C102H178N4O47
			24	Ganglioside GT1c (d18:1/24:0)	C102H178N4O47
740.03	$[M-2H]^{2-}$	1481.896996	95	Ganglioside GM2 (d18:1/25:0)	C74H135N3O26
746.03	$[M-2H]^{2-}$	1493.933333	94	Ganglioside GM2 (d18:1/26:1(17Z))	C75H135N3O26
	$[M-3H]^{3-}$	2241.213089	45	Ganglioside GT1b (d18:0/26:0)	C104H184N4O47
				Ganglioside GT1c (d18:0/26:0)	C104H184N4O47
747.03	$[M-2H]^{2-}$	1495.948982	84	Ganglioside GM2 (d18:1/26:0)	C75H137N3O26
749.03	$[M-2H]^{2-}$	1499.871175	125	Ganglioside GD3 (d18:1/20:0)	C72H129N3O29
750.03	$[M-2H]^{2-}$	1501.886825	85	Ganglioside GD3 (d18:0/20:0)	C72H131N3O29
758.03	$[M-2H]^{2-}$	1517.845354	151	Ganglioside GM1 (18:1/16:0)	C71H127N3O31
762.03	$[M-2H]^{2-}$	1525.886825	123	Ganglioside GD3 (d18:1/22:1(13Z))	C74H131N3O29
763.03	$[M-2H]^{2-}$	1527.902475	112	Ganglioside GD3 (d18:1/22:0)	C74H133N3O29