Tissue Imaging with New MALDI-TOF(TOF) Instrumentation

Presented at MSIA 2013

Vanderbilt University

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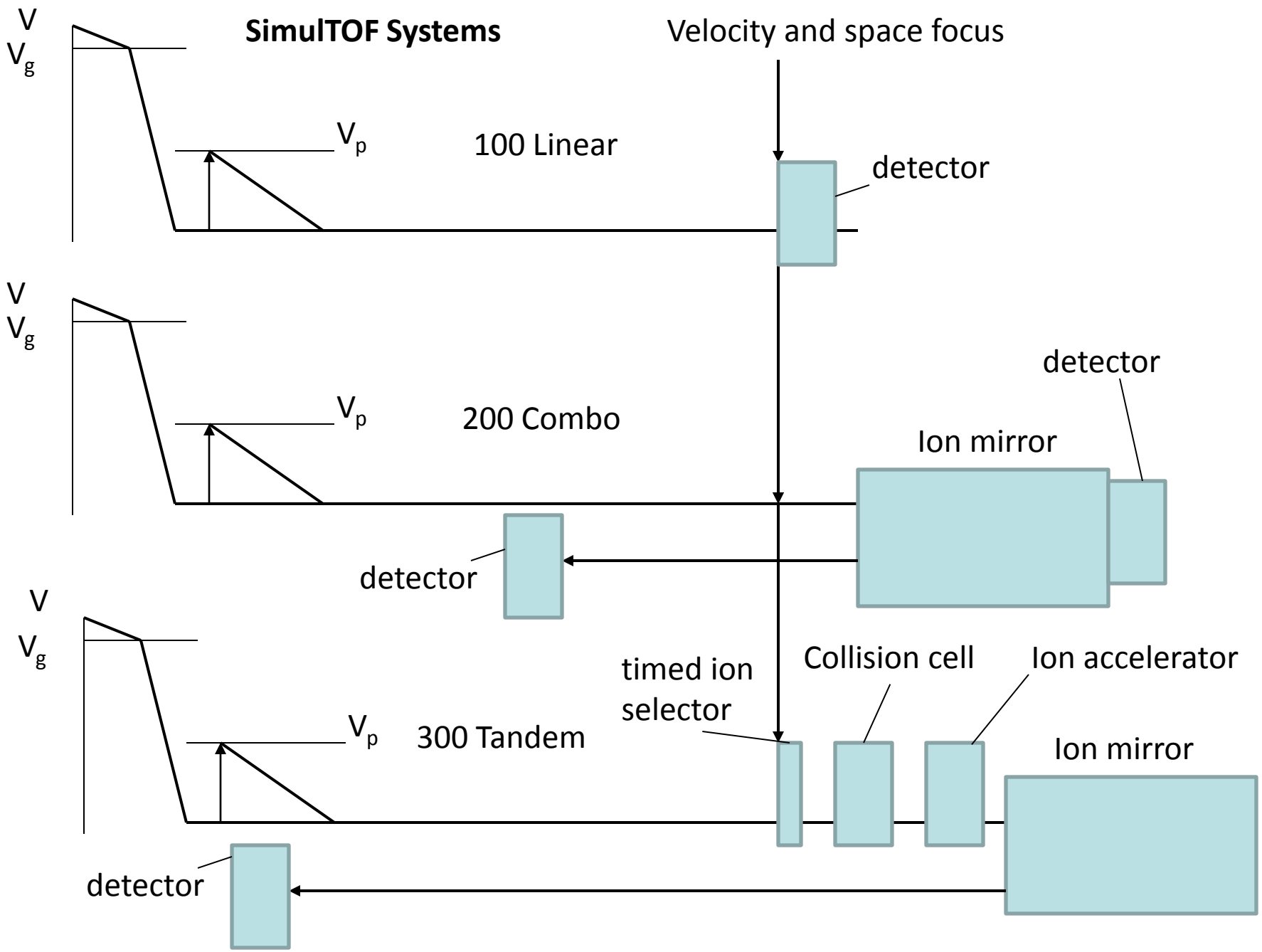
Outline

- Design Philosophy
- Modular Approach
- Technical Advances
  - 5 kHz laser
  - Efficient Ions Optics
  - Improved time focusing
  - Fast and efficient high mass detector
  - High resolution precurser selection and multiplexing for TOF-TOF
- Summary of performance
- Application to tissue imaging


SimulTOF Systems
Velocity and space focus
V
V_g
V_p
100 Linear
detector
200 Combo
Ion mirror
300 Tandem
timed ion selector
Collision cell
Ion accelerator

SimulTOF Systems

Guiding Innovations in Science for Life

Commercial products introduced at ASMS 2012

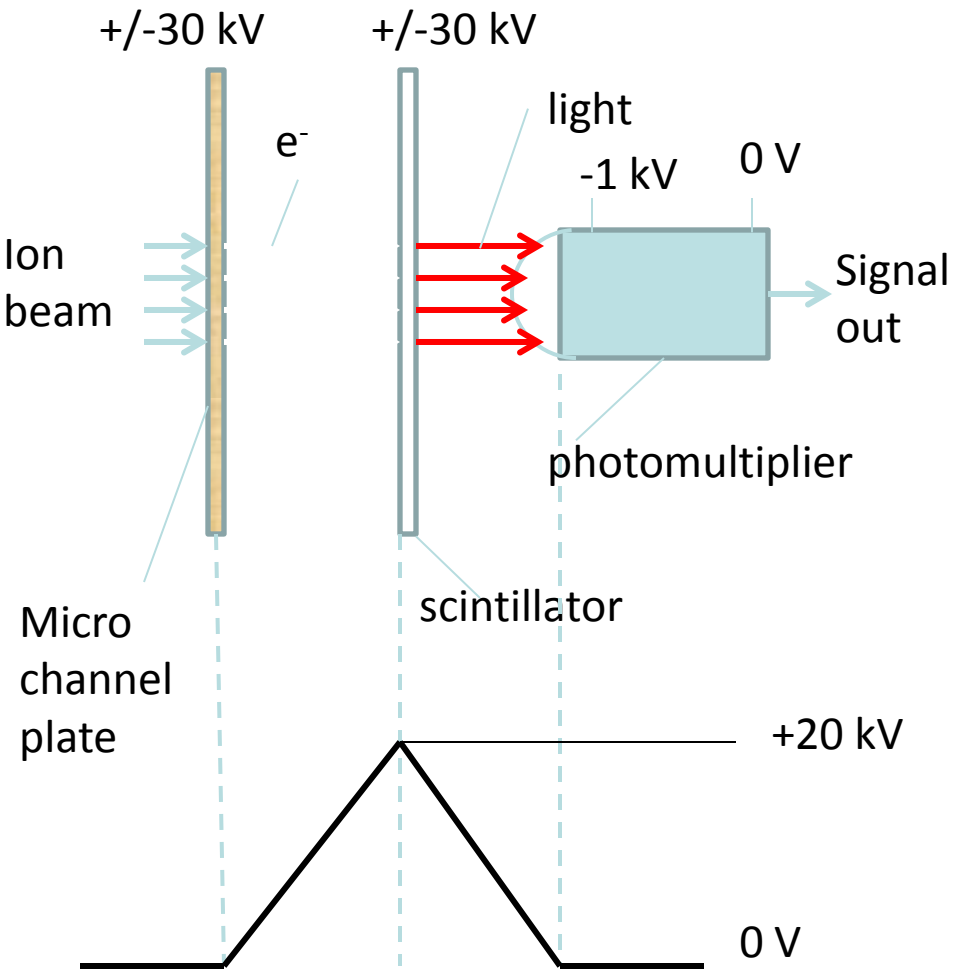
Design Philosophy

- We have employed a modular design philosophy, where a number of systems/modules are common to all three initial products.
  - Reduces complexity
  - Delivers advantages for manufacturing and supply chain.
  - Facilitates our simultaneous introduction of multiple products
  - Enables options that respond to the needs of specific customers and applications.
- Deliver a robust/reliable product that consistently performs as intended.
- Design for manufacturability and serviceability
- Build on decades of experience designing, building, and maintaining instruments, particularly mass spectrometers
- Keep the manufacturing process as simple and straightforward as possible
- Ensuring adequate access for testing, troubleshooting, and repair.

Technical Advances in SimulTOF 100 Linear MALDI-TOF

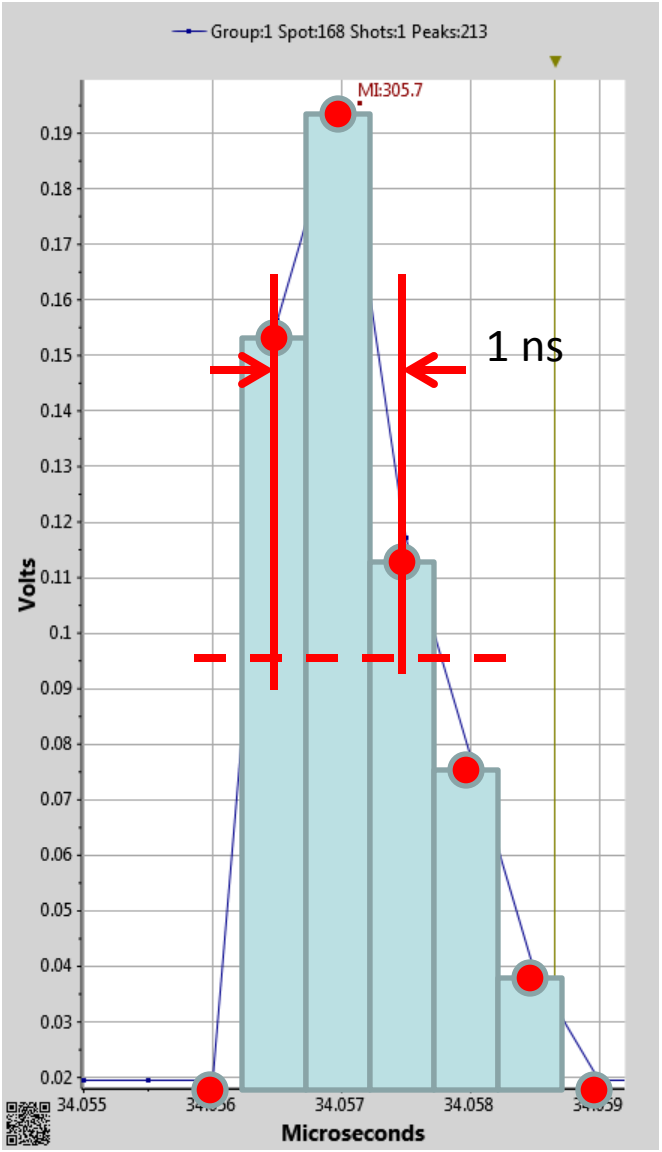
- 20 kV source and novel high speed, high mass detector provides very high sensitivity, resolving power, and accuracy over broad mass range
- isotopic resolution below 3 kDa, and isotopic envelope resolution for high masses to 50 kDa
- High laser rate (5 kHz) and high acquisition rate (up to 50 spectra/s) makes tissue imaging practical
- Proprietary ion optics and high laser rate provide sensitivity and dynamic range limited only by chemical noise
- Resolving power>4000 @2465 Da
- Resolving power>2m for full range from 100 to 2000 Da
- Mass range 100Da-500 kDa
- Mass error <30 ppm across the plate over the mass range 300-30,000 Da with single peak automatic calibration
- Dynamic range up to100,000

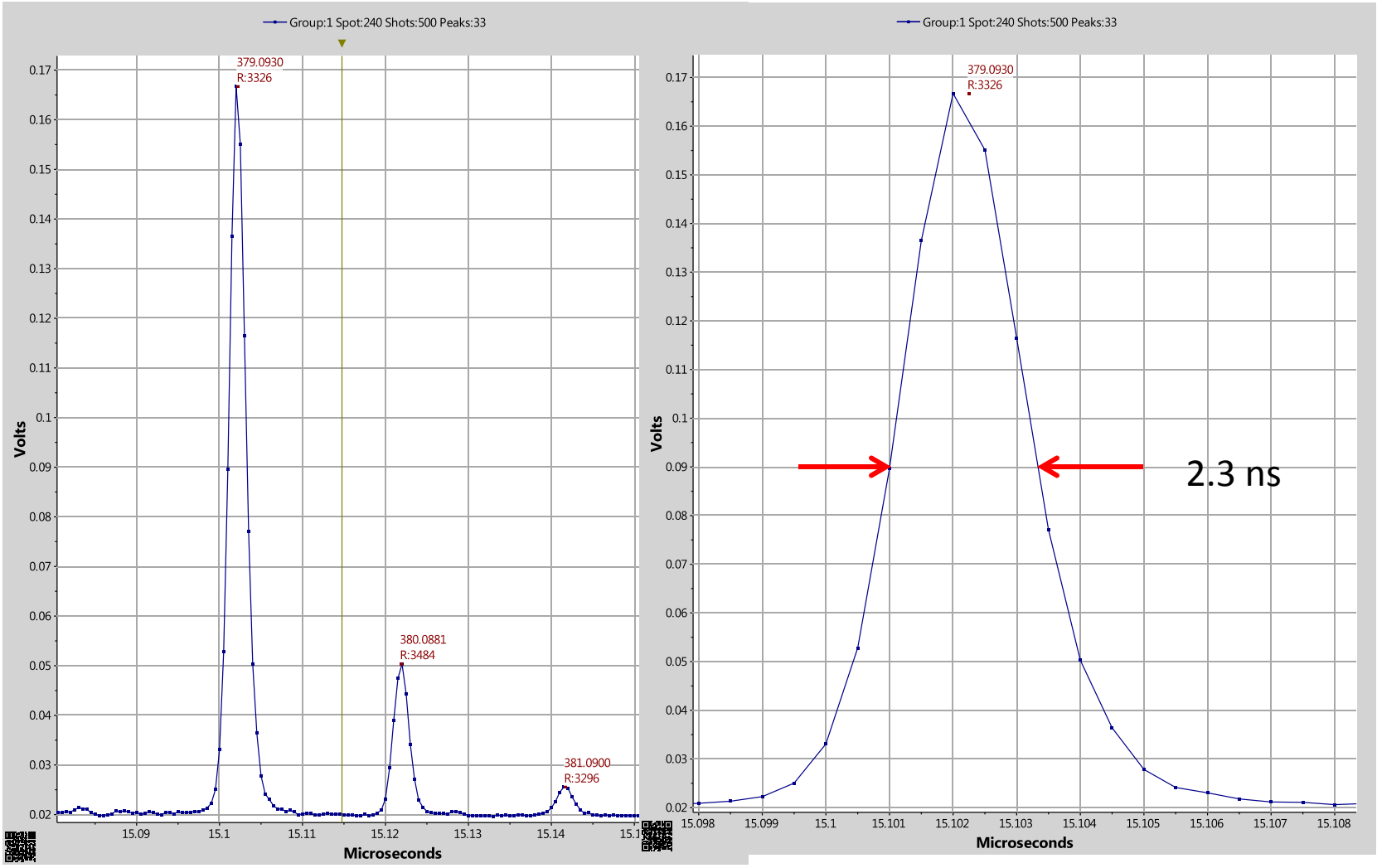
Photonis detector



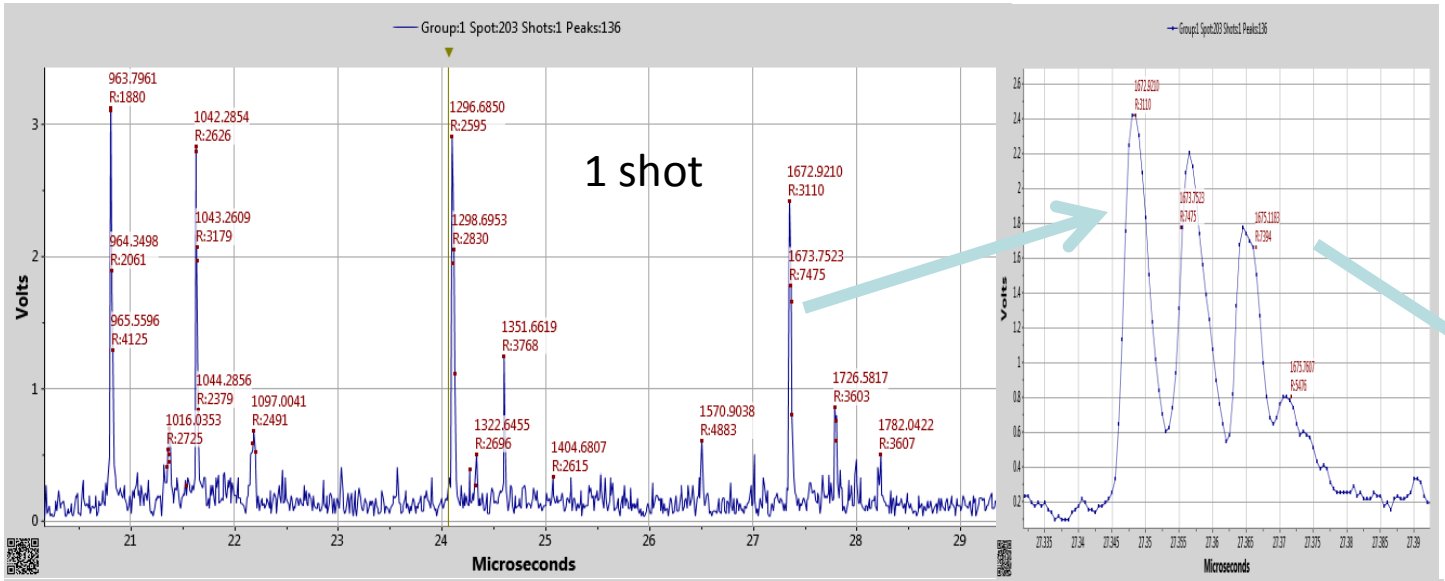
Potential diagram for linear detector

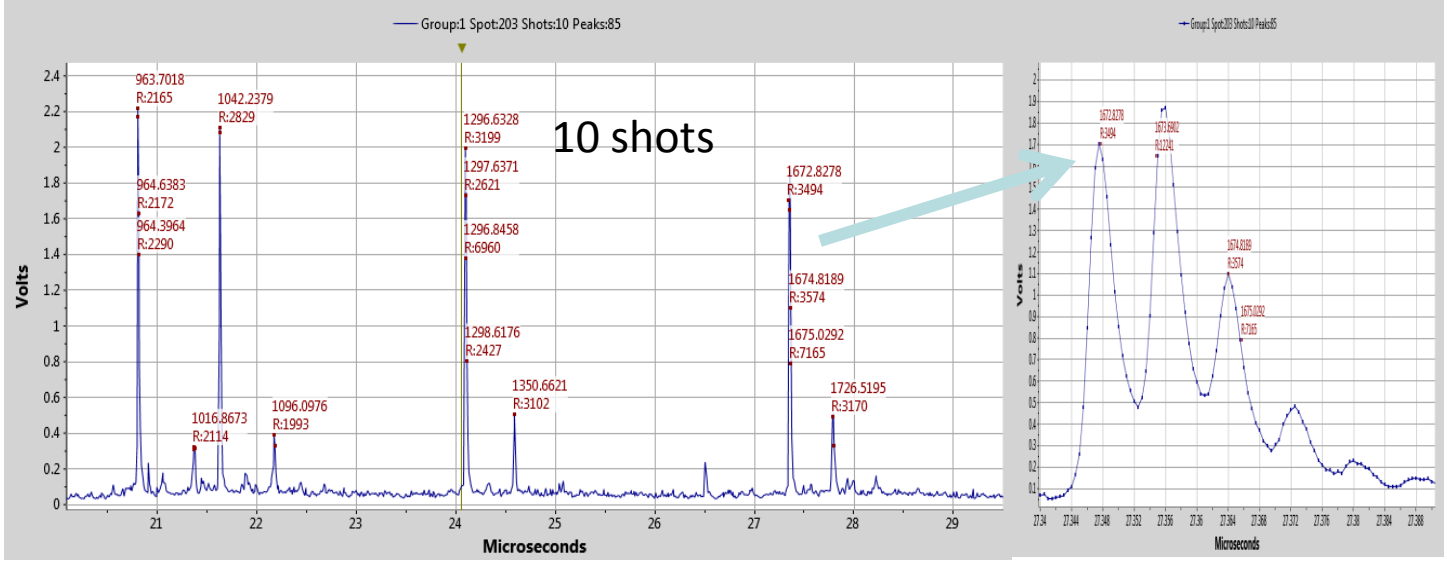
Typical single ion pulse with fast scintillator





Spectrum of α-cyano matrix dimer measured in SimulTOF 100 Linear MALDI-TOF
At 15 kV energy using Photonis fast hybrid detector





~20,000 ions detected
<240,000 Molecules consumed

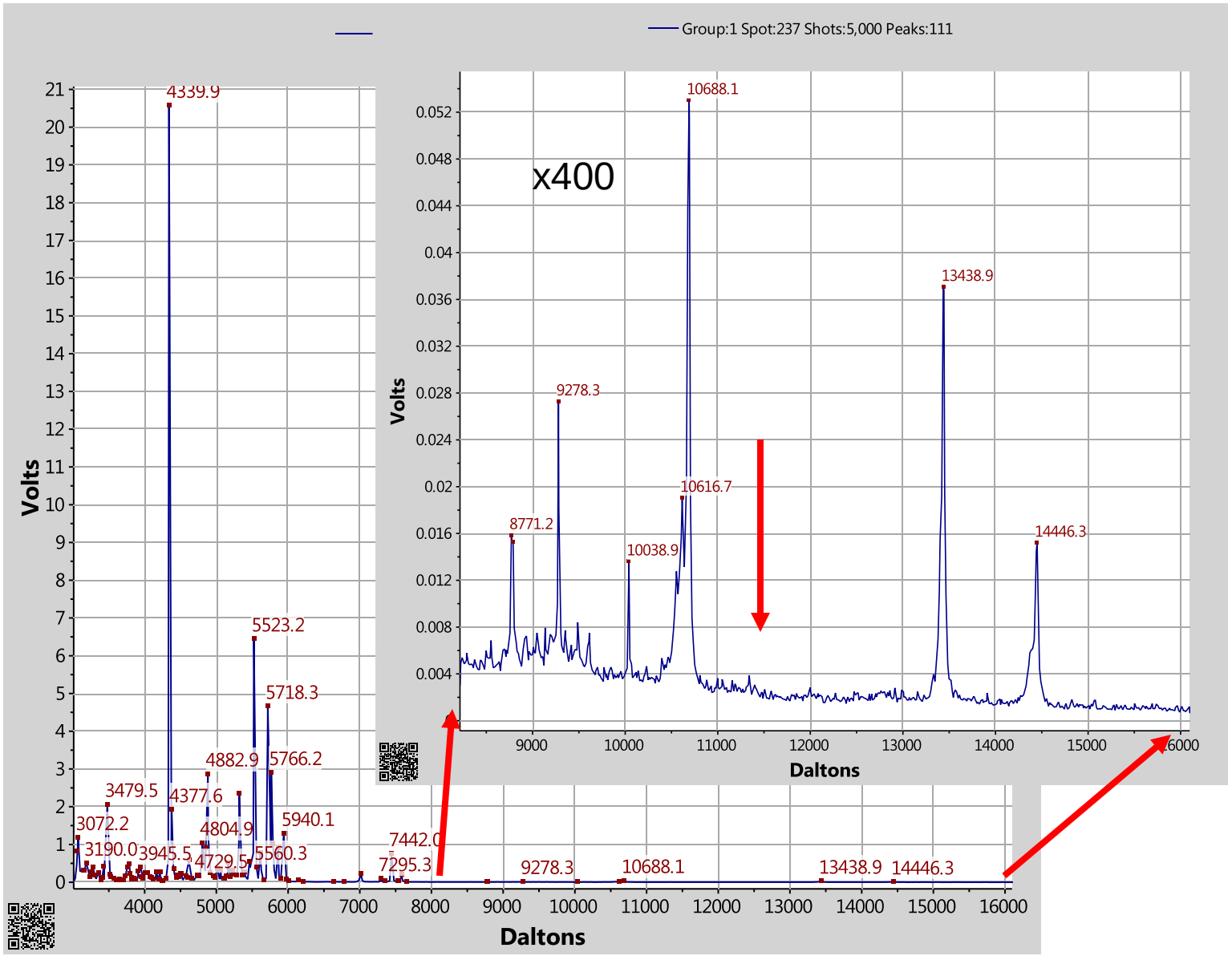
Mixture of peptide standards, 100 femtomole/µL, 2.5 mm spot
Laser spot ca. 50 µm, fluence 1.3x threshold, 40 attomole/spot
<1% consumed/laser shot=240,000 molecules

Applications of MALDI-TOF

- Pathogen Identification
- Cancer typing directly from serum, tissue extracts, and other bodily fluids
- Tissue imaging
  - Proteins for cancer typing
  - Small molecules for drug disposition
- Biomarker Identification and Validation
  - Mass Spec Immunoassay
  - Peptide quantitation (SISCAPA and others)
- Clinical assays of biomarkers for diagnosis and treatment
- QC of synthetic olignucleotides, peptides, proteins and small molecules

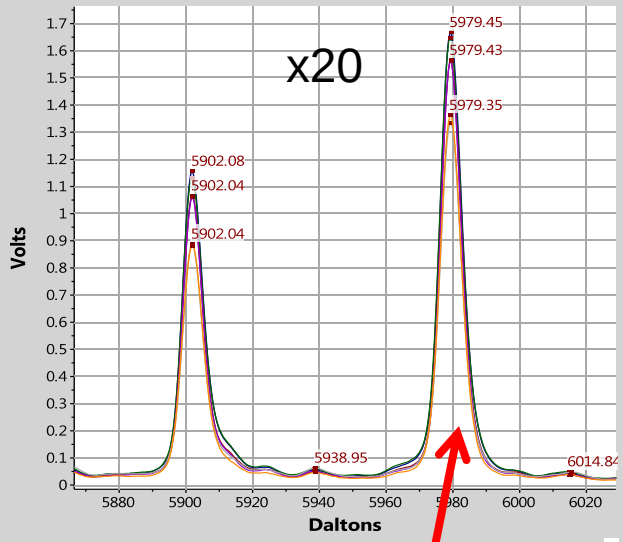
Performance of SimulTOF 100 Linear for Complex Biological Samples

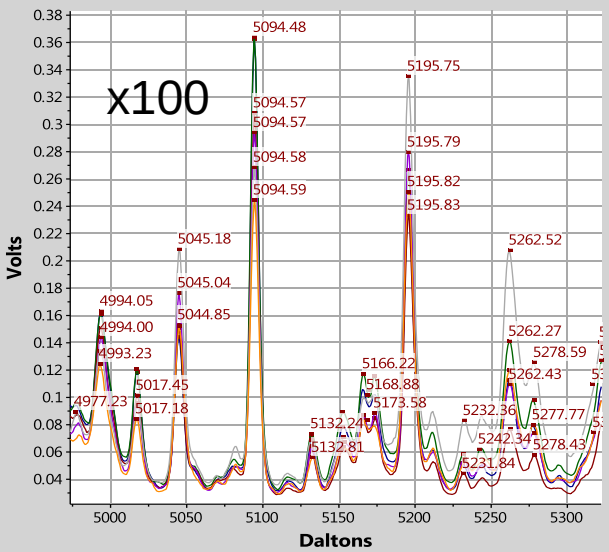
- Human Saliva
  - Diluted 5:1 in HCCA matrix and spotted on MALDI plate
  - 17 spots analyzed in linear mode over mass range 3000-3000
  - 5000 shots summed to produce spectra
  - At 5 kHz acquisition time is 1 second
- Serum sample
  - diluted 1:10 in sinipinic acid
  - 10 spots analyzed in linear mode over mass range 3000-3000
  - 10000 shots summed to produce spectra
  - At 5 kHz acquisition time is 2 seconds
- Oligonucleotides

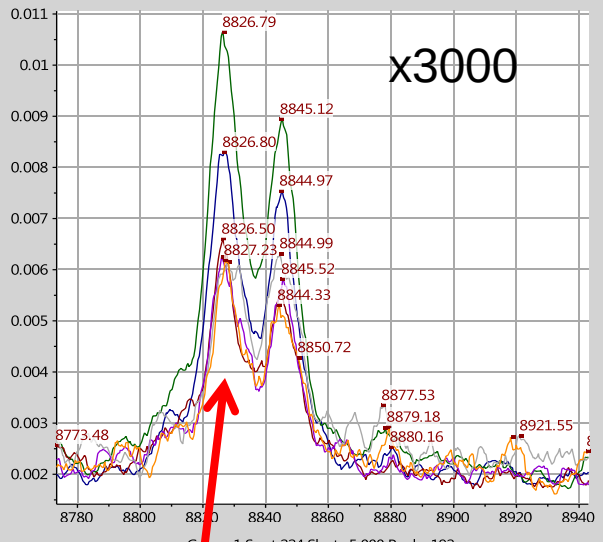


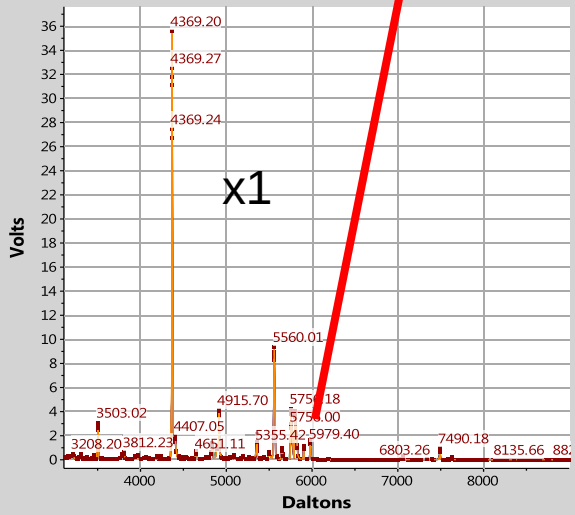
Spectrum from one spot (3 mm dia) for 1:5 dilution of saliva sample in HCCA 5000 shots

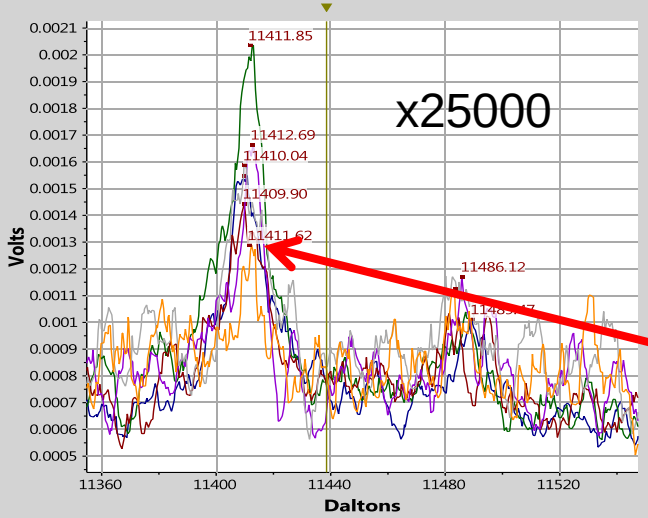
Examples of Superimposed Spectra from 6 spots across the plate illustrating dynamic range and mass accuracy obtained in saliva analysis

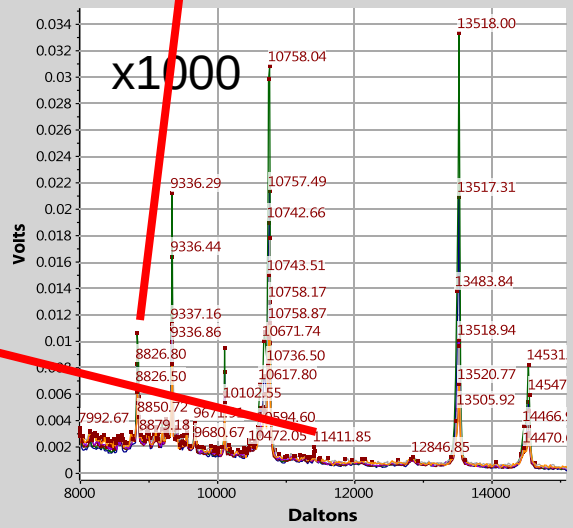




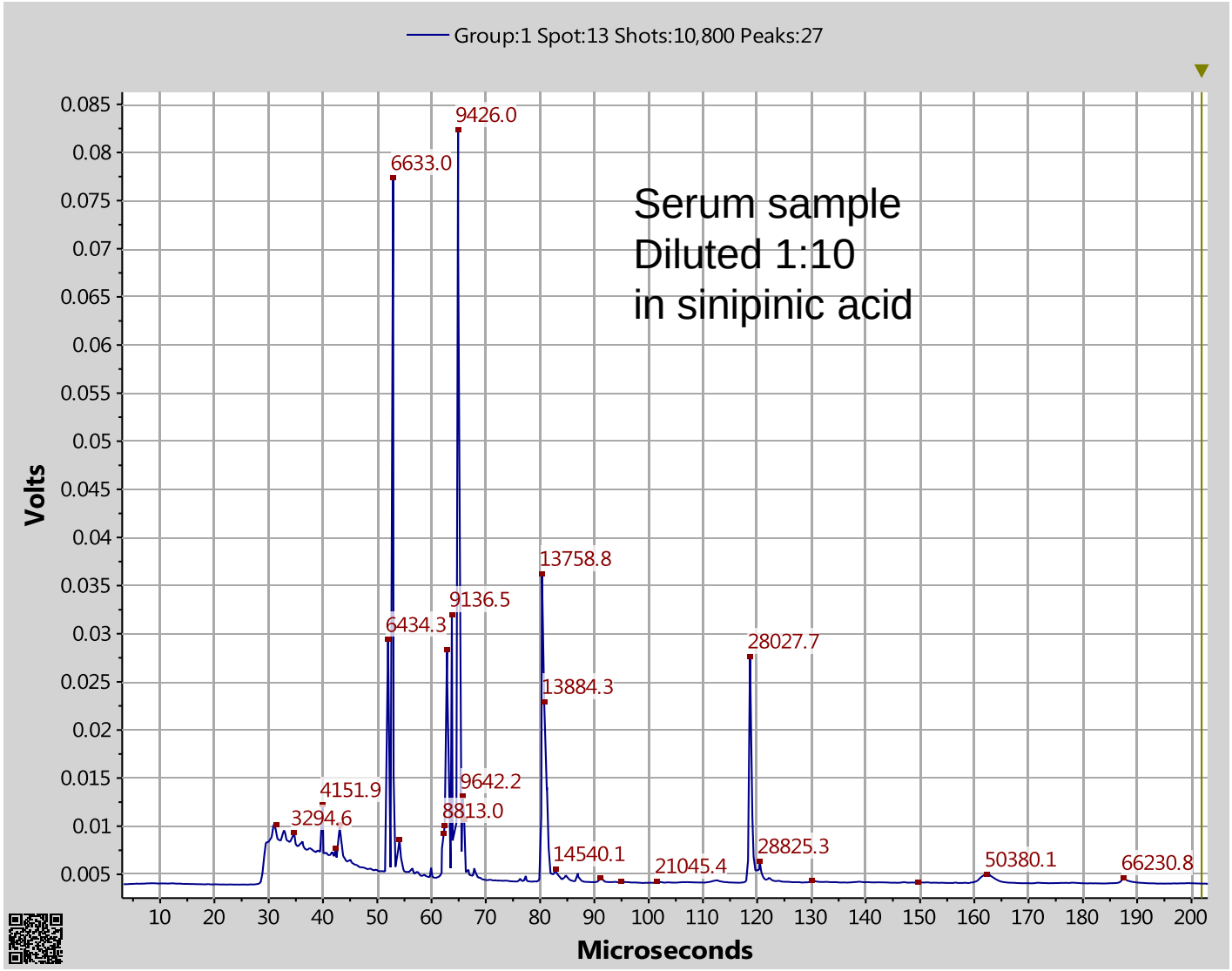






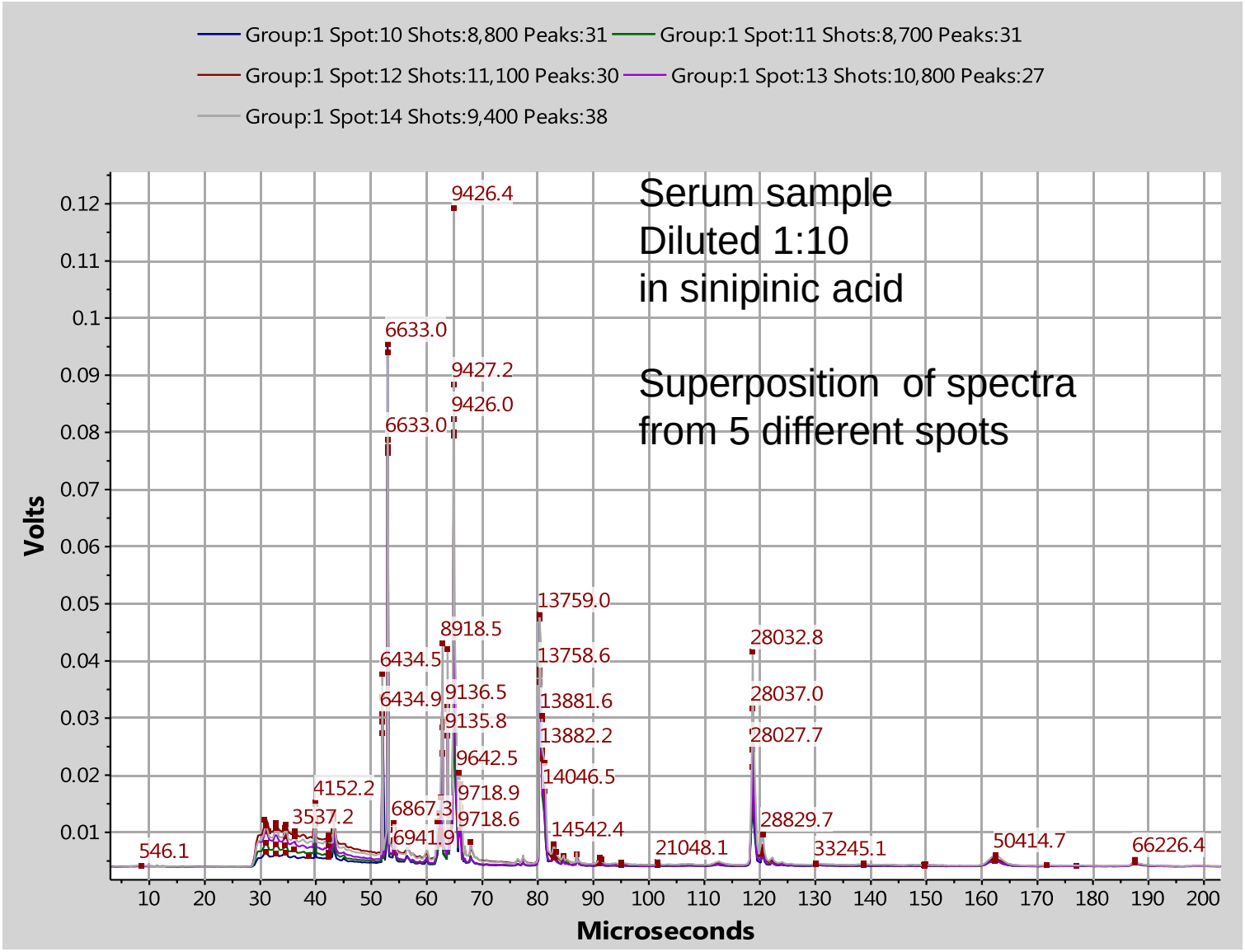


Sensitivity, Dynamic Range, and Reproducibility are Key Metrics

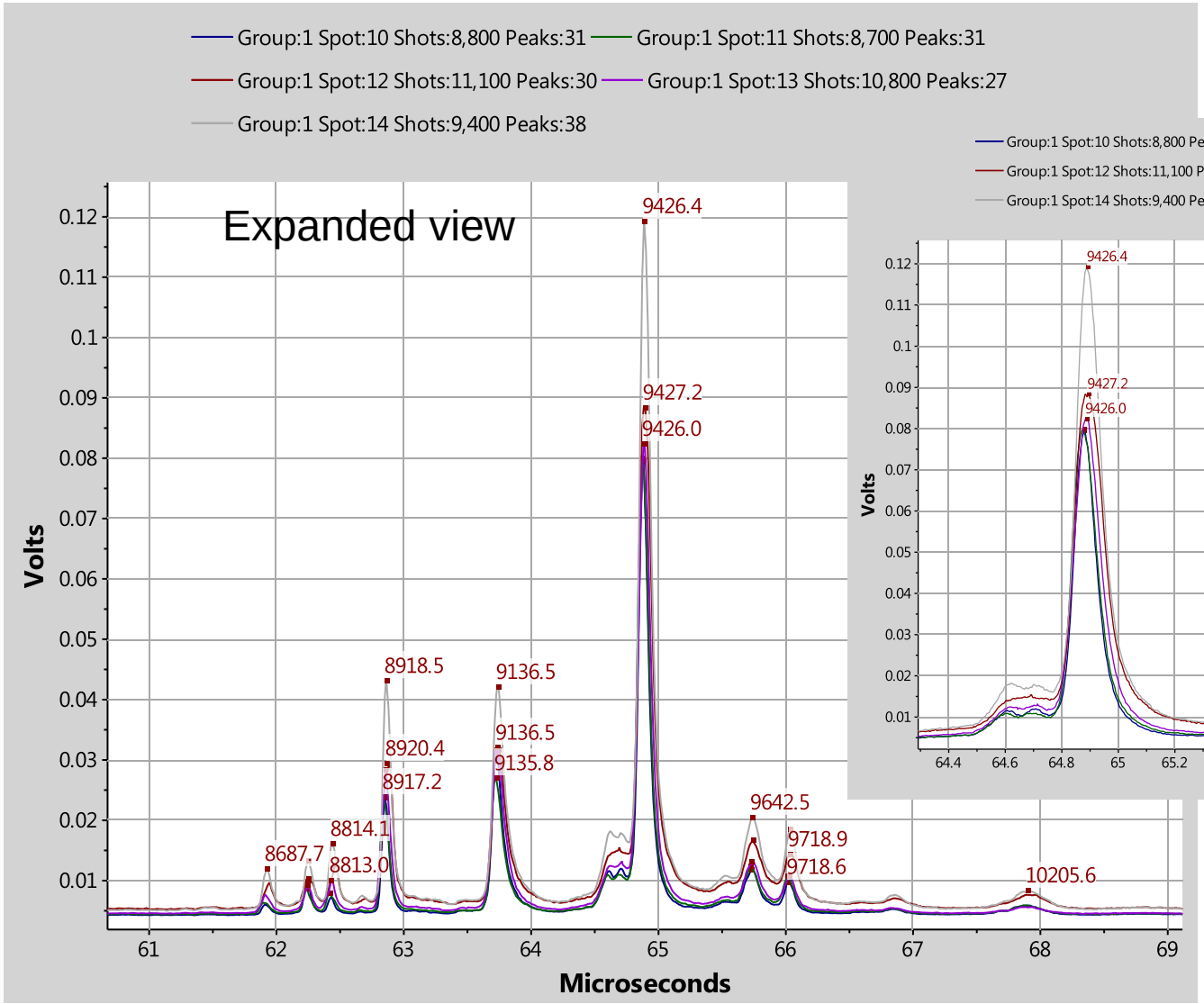


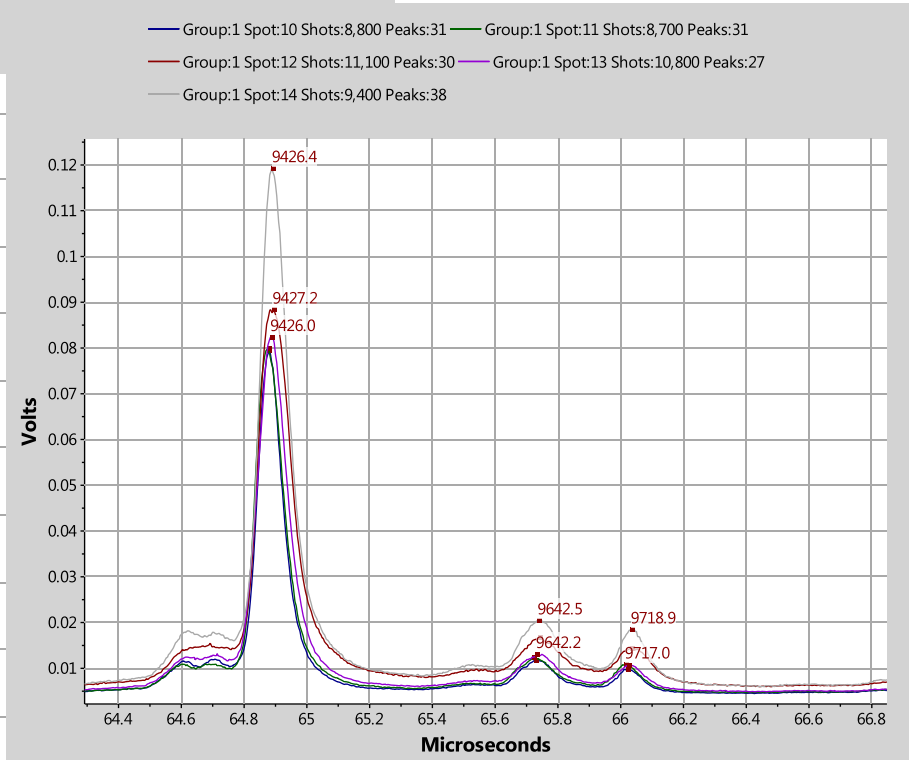
Data from SimulTOF 100 Linear

Sensitivity, Dynamic Range, and Reproducibility are Key Metrics

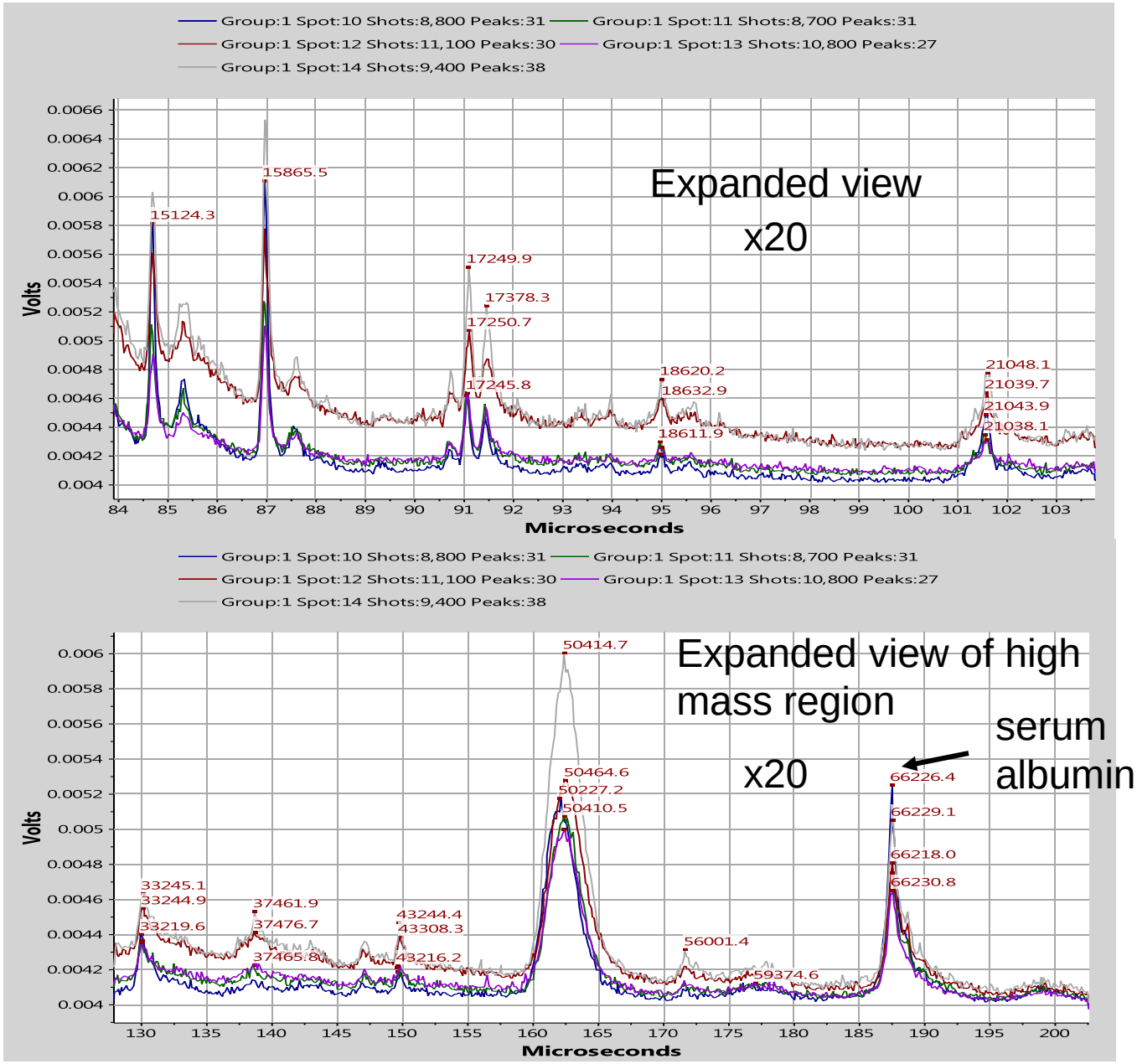


Data from SimulTOF 100 Linear



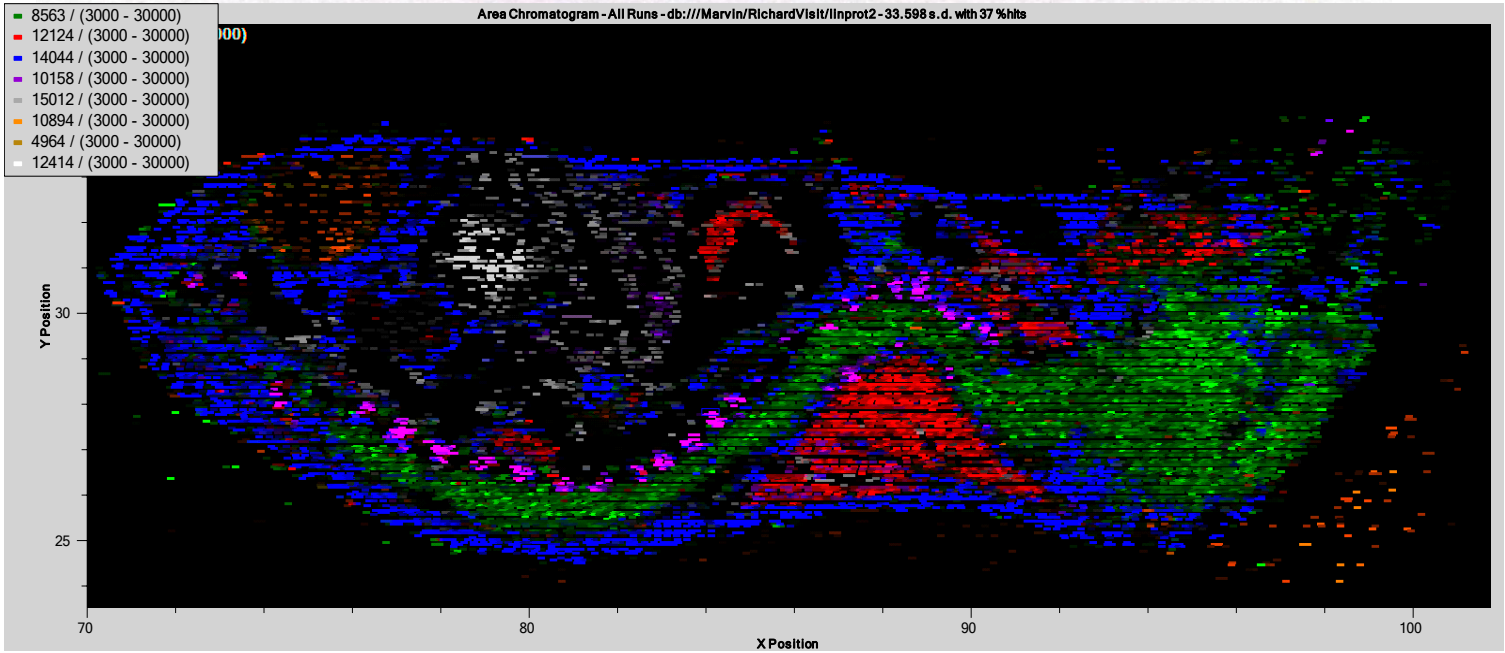


These are raw data. No normalization, background subtraction, smoothing or other data processing has been employed.

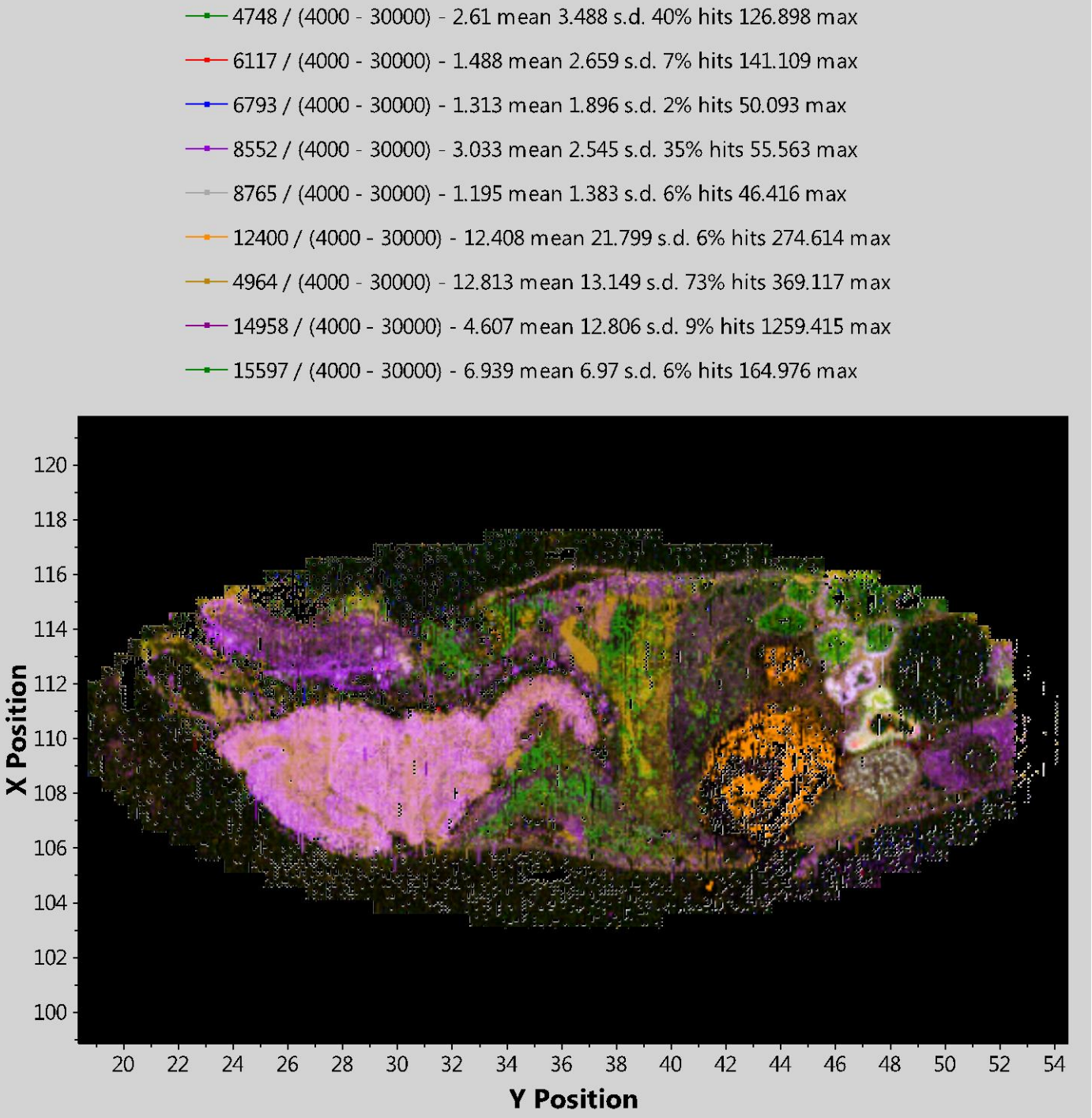

Group:1 Spot:10 Shots:8,800 Peaks:31
Group:1 Spot:11 Shots:8,700 Peaks:31
Group:1 Spot:12 Shots:11,100 Peaks:30
Group:1 Spot:13 Shots:10,800 Peaks:27
Group:1 Spot:14 Shots:9,400 Peaks:38
Expanded view
x20
15865.5
15124.3
17249.9
17378.3
17250.7
17245.8
18620.2
18632.9
18611.9
21048.1
21039.7
21043.9
21038.1
Volts
Microseconds
Group:1 Spot:10 Shots:8,800 Peaks:31
Group:1 Spot:11 Shots:8,700 Peaks:31
Group:1 Spot:12 Shots:11,100 Peaks:30
Group:1 Spot:13 Shots:10,800 Peaks:27
Group:1 Spot:14 Shots:9,400 Peaks:38
Expanded view of high mass region
x20
serum albumin
50414.7
50464.6
50227.2
50410.5
66226.4
66229.1
66218.0
66230.8
33245.1
33244.9
33219.6
37461.9
37476.7
37465.8
43244.4
43308.3
43216.2
56001.4
59374.6
Volts
Microseconds

Conclusions

- Resolving power 500-1000 over wide range is routine
- Normalization to TIC removes most of amplitude variation
- Each spot will yield up to 100,000 shots without degrading resolving power or accuracy and giving dynamic range limited only by chemical noise
- Results might be improved by multiple levels of dilution and use of alternative matrices
- Mass error <30 ppm across the plate over the full mass range with single peak automatic calibration
- Dynamic range up to100,000



Protein images of whole mouse pup at 5 kHz, 37 μJ/pulse, sinapinic acid matrix, 1 mm/s, 500 shots/sp, 50x100 μm pixels,100,000 spectra in 3 hours Prototype instrument



SimulTOF 200 in Linear Mode

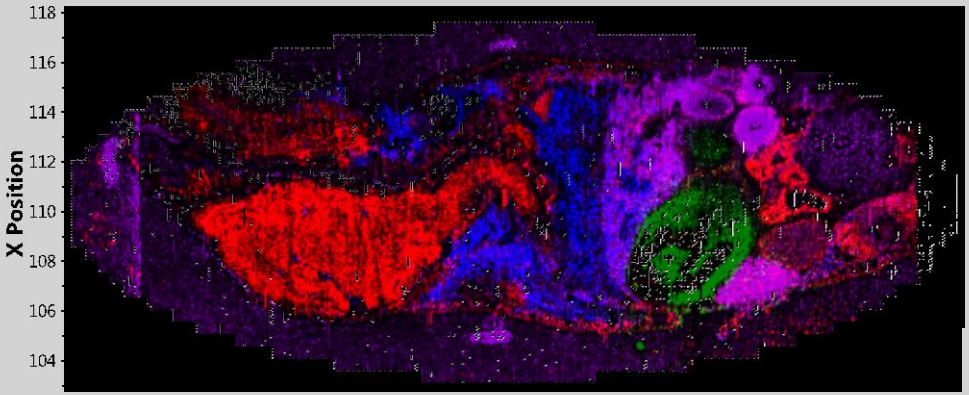
127,725 spectra

50x50 μm pixels

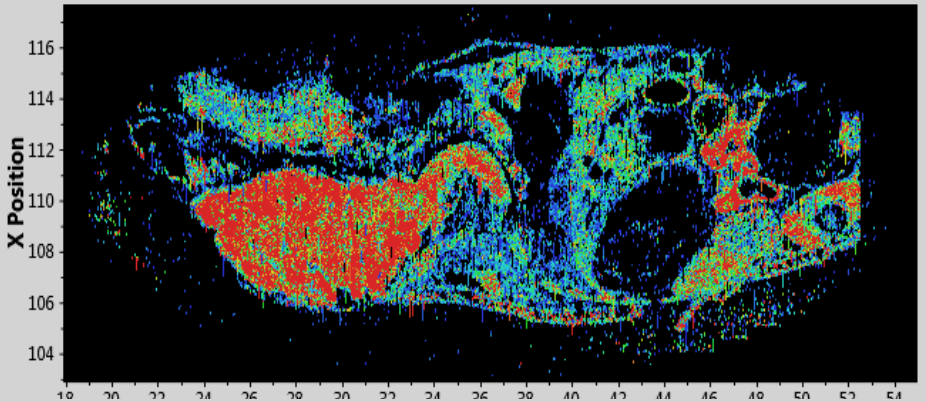
50 spectra/s at 5 kHz, 2.5 mm/s

45 minutes total

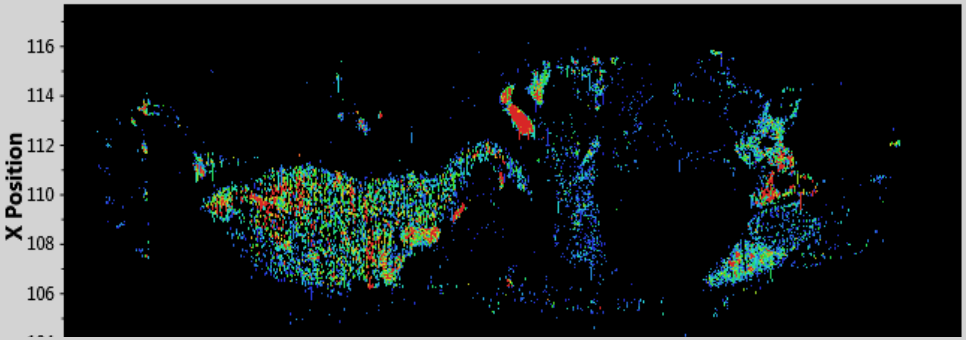
12400 - 9636.182 mean 21983.196 s.d. 12% hits 289433.15 max
8558 - 2200.302 mean 2429.649 s.d. 38% hits 41045.334 max
15597 - 4842.387 mean 5811.941 s.d. 16% hits 102862.254 max
3216 - 4110.117 mean 7587.706 s.d. 85% hits 176408.567 max



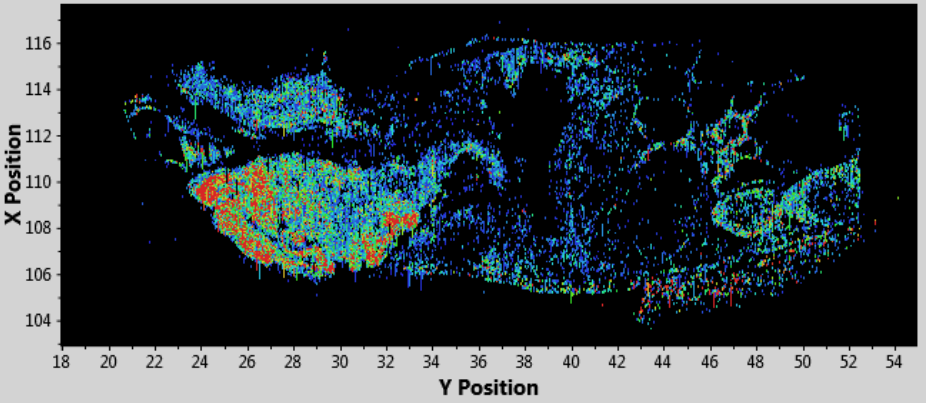
8552 - 2265.271 mean 2460.431 s.d. 35% hits 41045.334 max



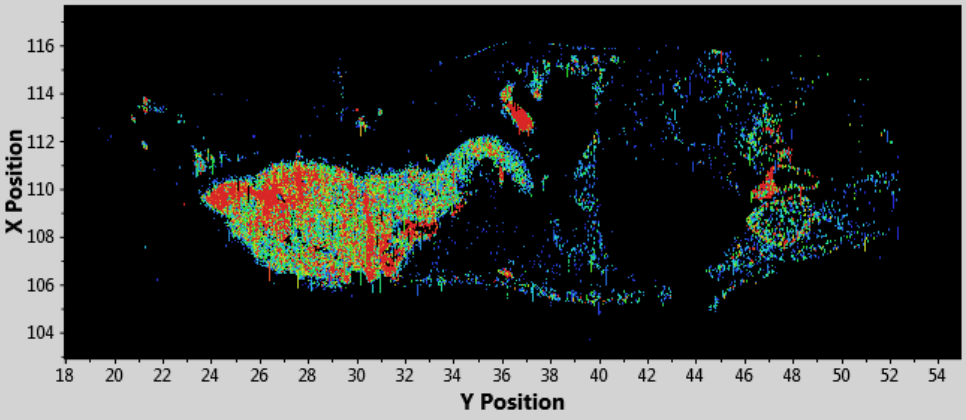
4965 - 7331.397 mean 12048.527 s.d. 46% hits 233517.324 max



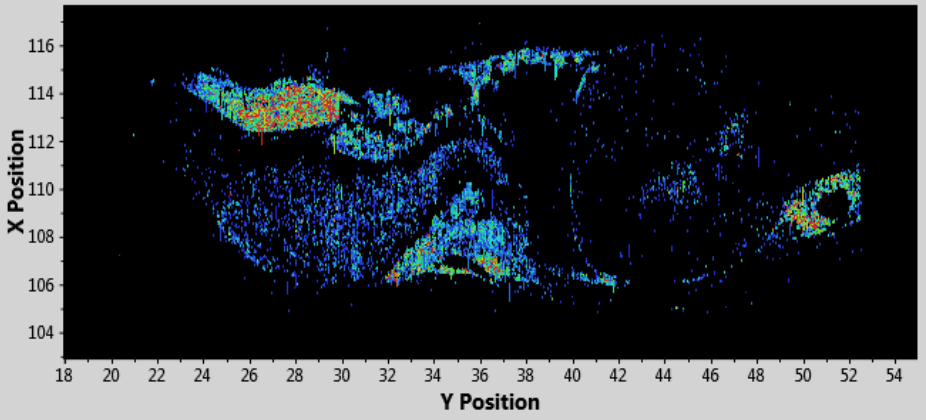
11323 - 1217.65 mean 1236.421 s.d. 17% hits 27203.462 max



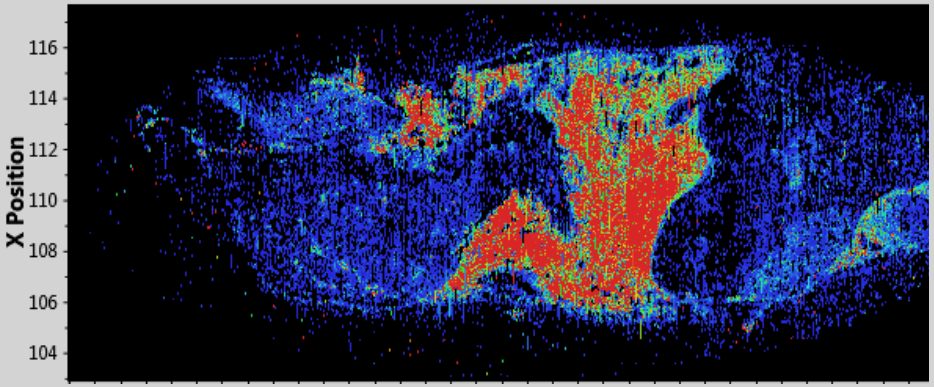
4939 - 4247.168 mean 5417.604 s.d. 36% hits 67053.465 max



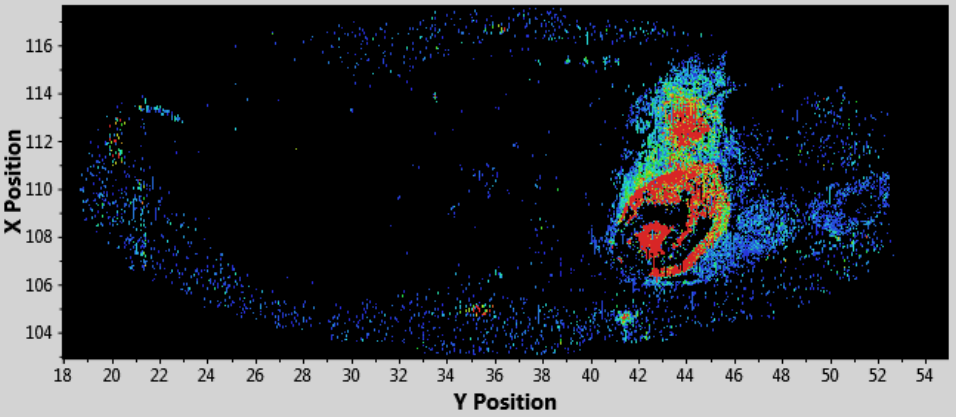
12112 - 1362.516 mean 1611.673 s.d. 9% hits 33609.098 max



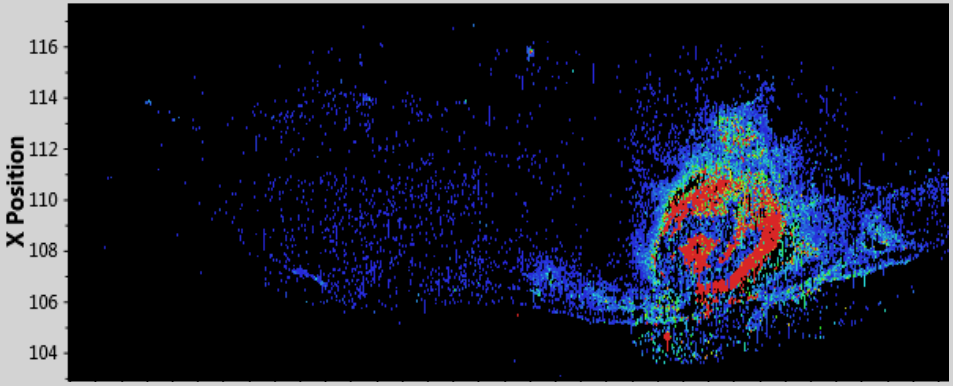
14800 - 16000 - 10097.358 mean 15807.881 s.d. 34% hits 293333.895 max



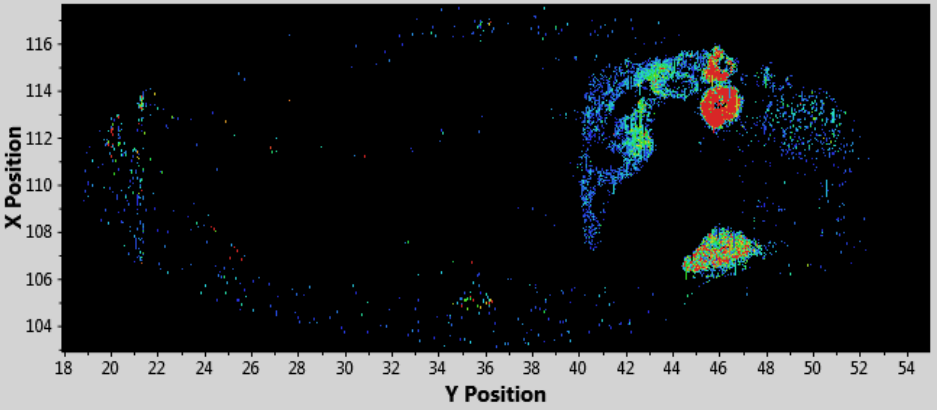
2788 - 8293.234 mean 17672.01 s.d. 35% hits 250070.744 max



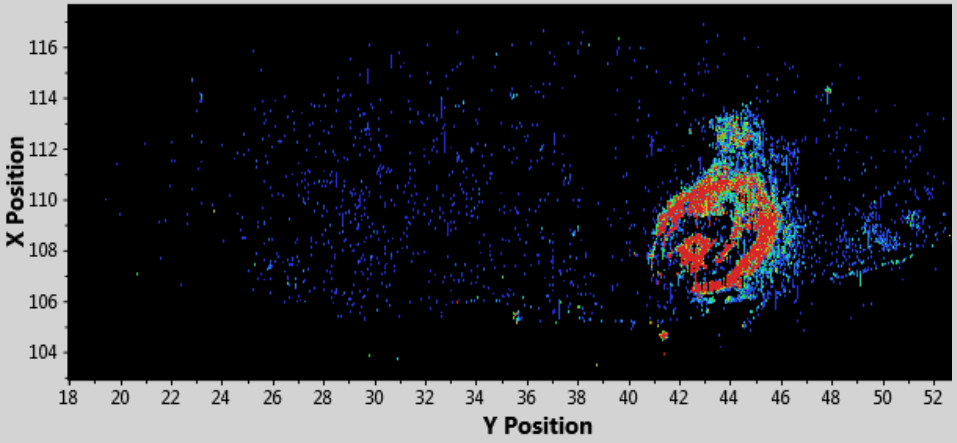
12400 - 9636.182 mean 21983.196 s.d. 12% hits 289433.15 max



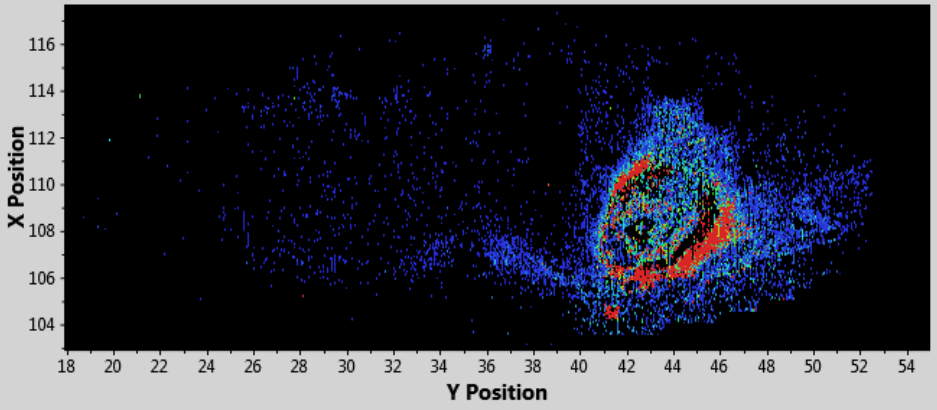
3217 - 4413.622 mean 8711.017 s.d. 50% hits 176408.567 max

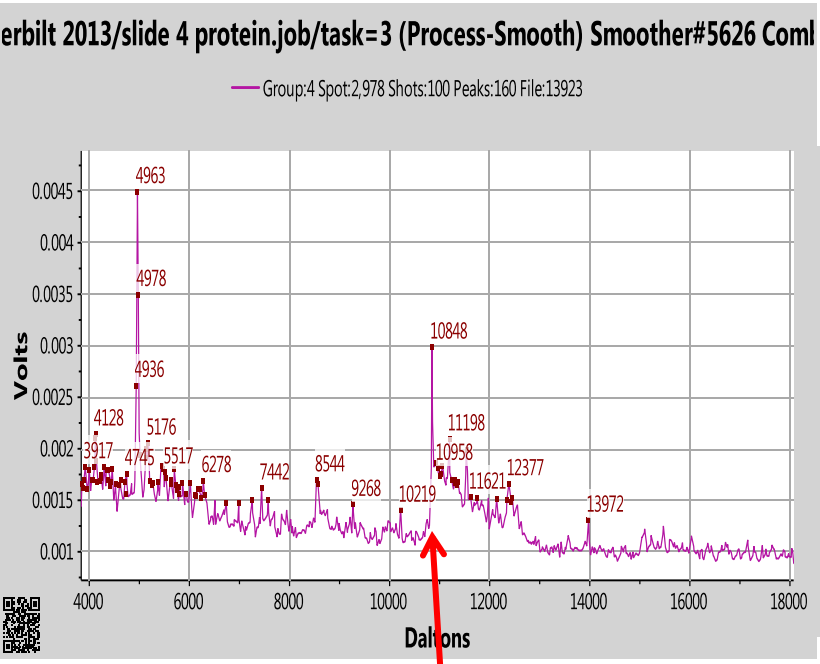


11189 - 4055.892 mean 6019.176 s.d. 6% hits 64021.172 max



12442 - 8515.541 mean 20473.812 s.d. 9% hits 320560.099 max

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Group:4 Spot:2,978 Shots:100 Peaks:160 File:13923
Volts
Daltons
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4978
4936
10848
11198
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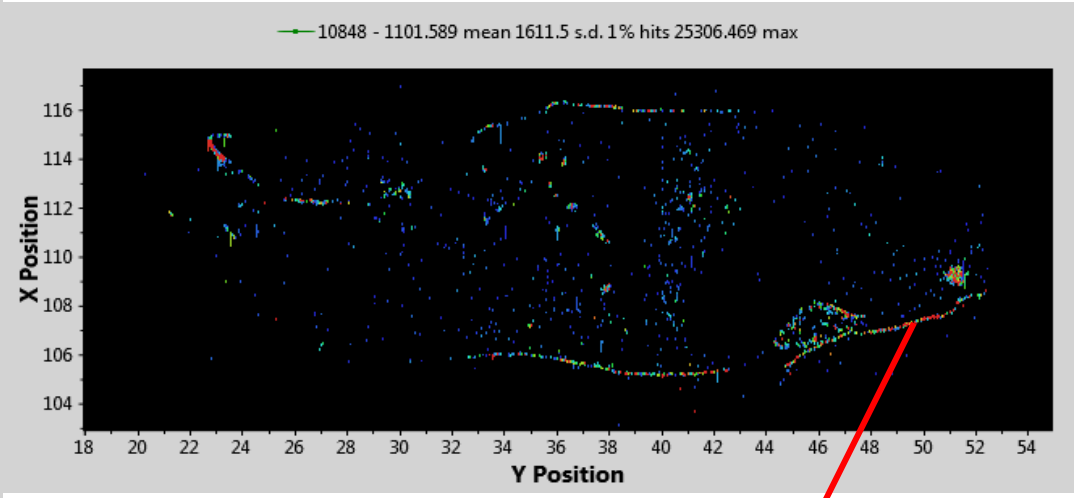
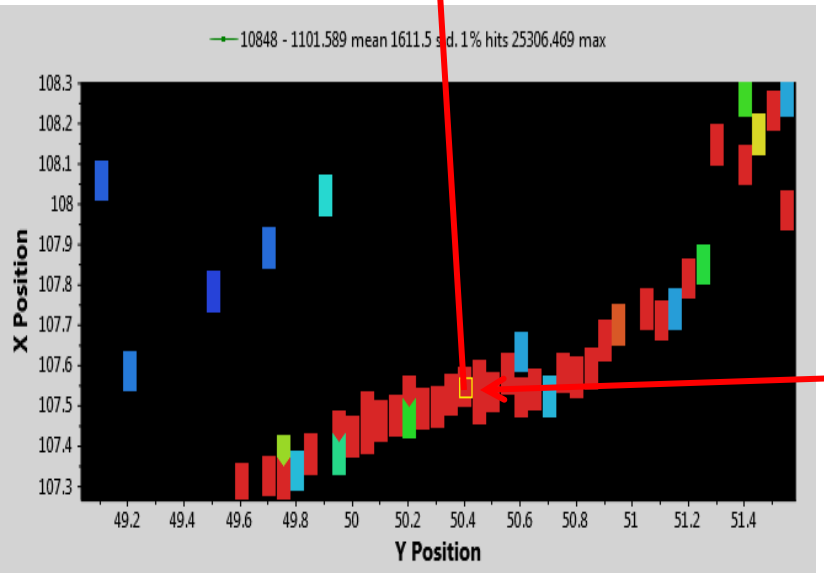
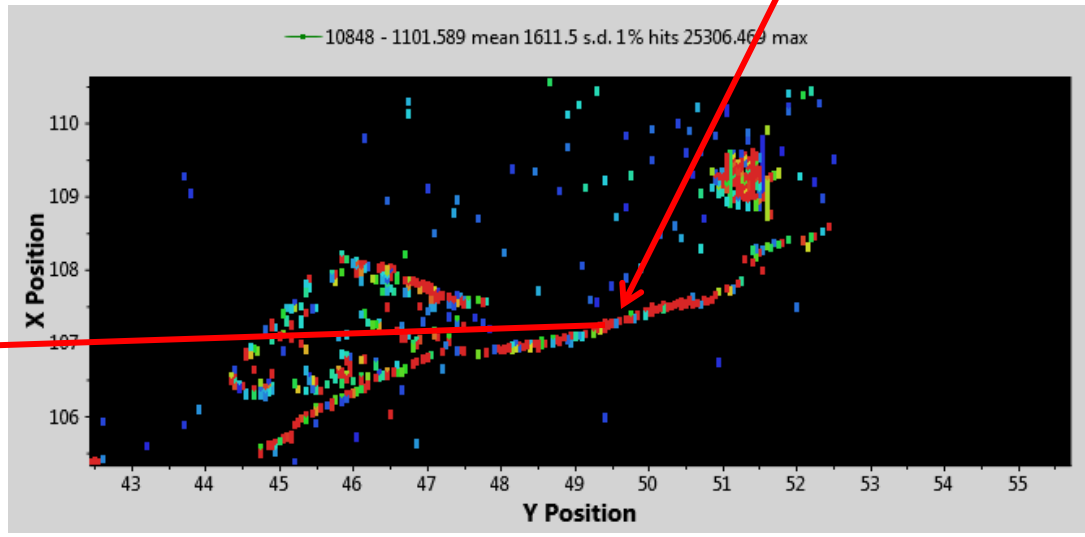
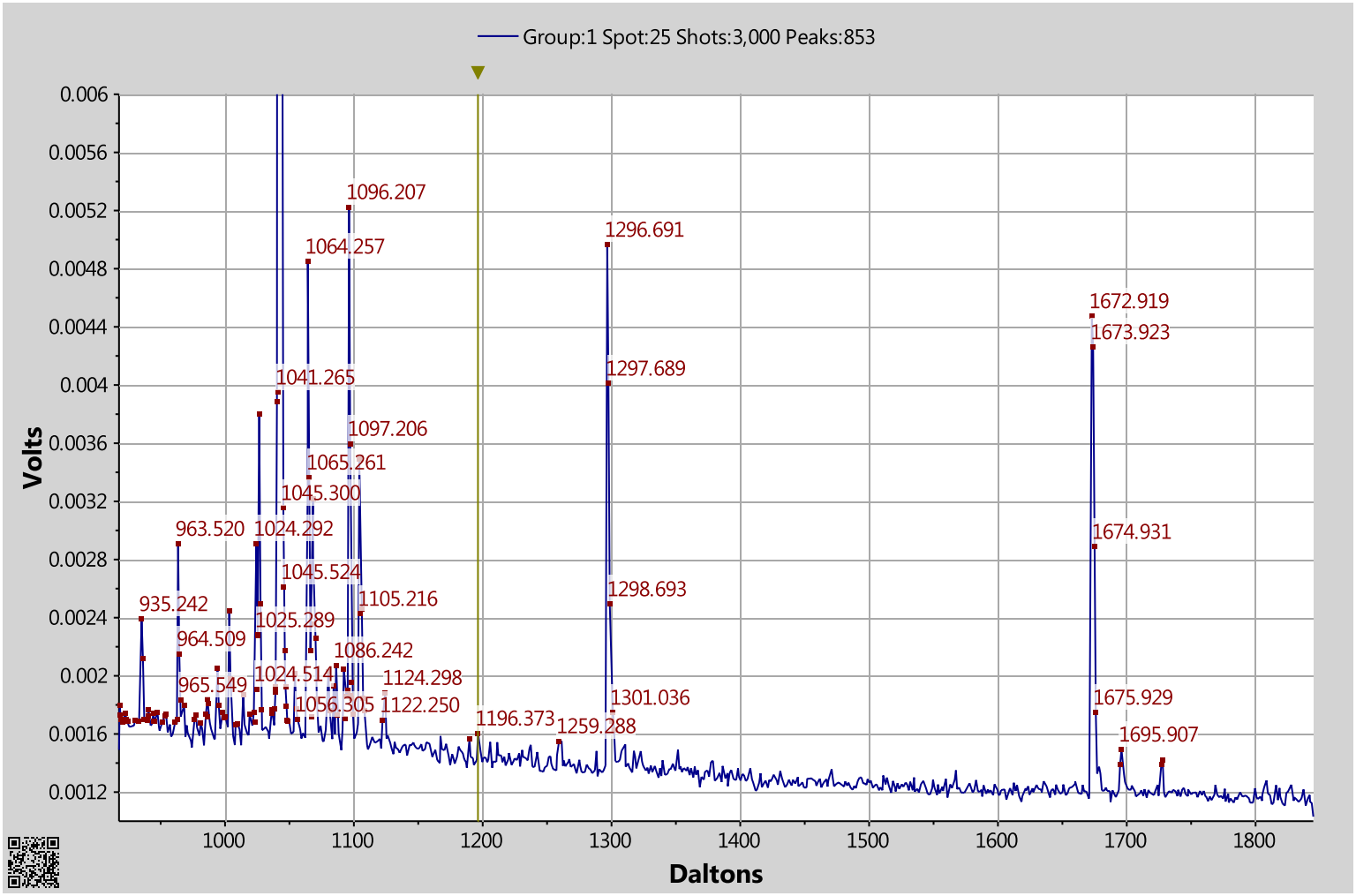

10848 - 1101.589 mean 1611.5 s.d. 1% hits 25306.469 max
X Position
Y Position


10848 - 1101.589 mean 1611.5 s.d. 1% hits 25306.469 max
X Position
Y Position


10848 - 1101.589 mean 1611.5 s.d. 1% hits 25306.469 max
X Position
Y Position

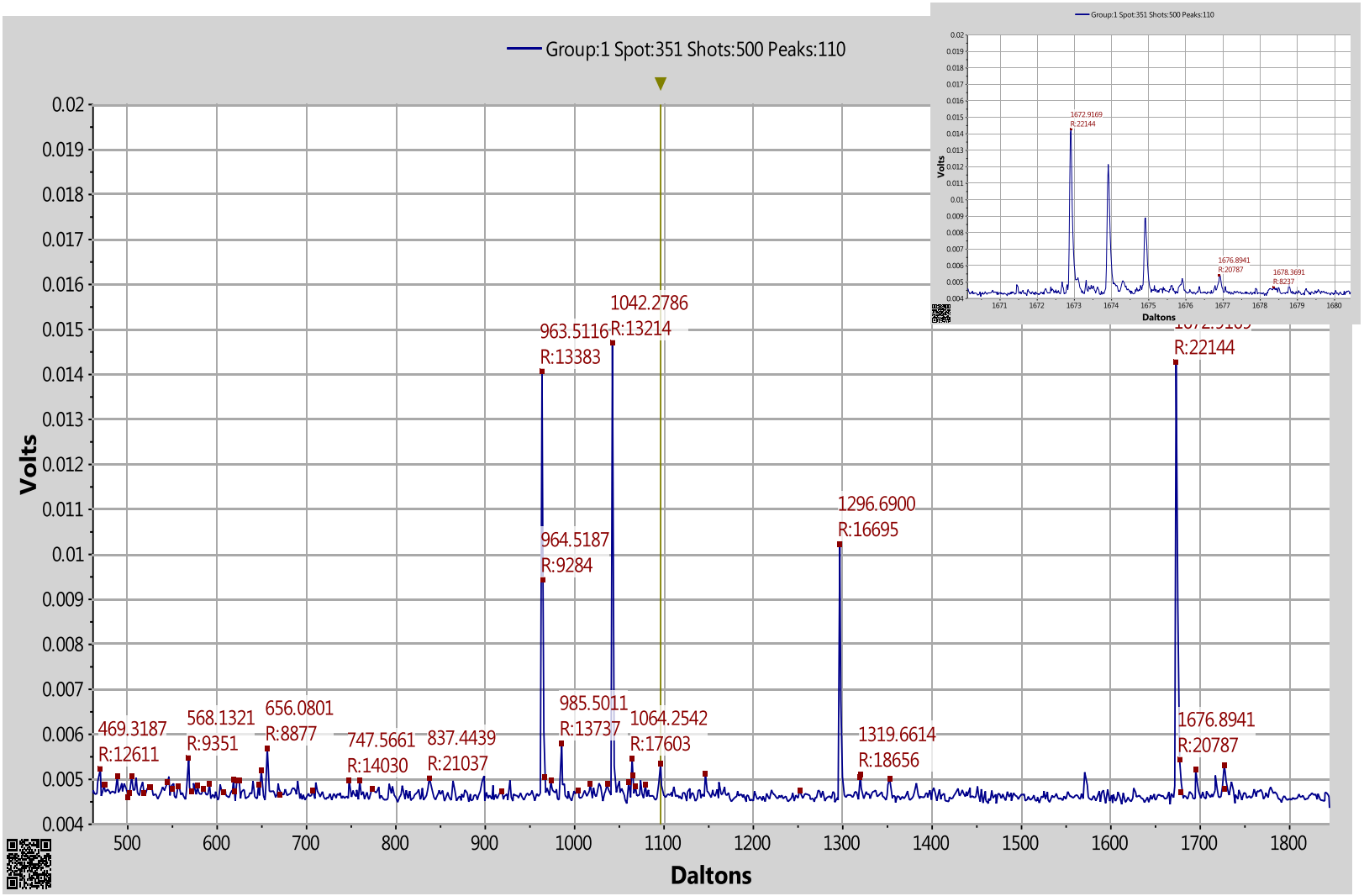
Specifications for SimulTOF 200 Combo MALDI-TOF

- Linear Analyzer (identical to 100)
  - 20 kV source and novel high speed, high mass detector provides very high sensitivity, resolving power, and accuracy over broad mass range
  - isotopic resolution below 3 kDa, and isotopic envelope resolution for high masses to 50 kDa
  - High laser rate (5 kHz) and high acquisition rate (up to 50 spectra/s) makes tissue imaging practical
  - Resolving power>5000 @2465 Da
  - Resolving power>2m for full range from 100 to 2500 Da
  - Mass range 100Da-500 kDa
- Reflecting Analyzer
  - Resolving power >20,000 at focus mass, >10,000 over range 800-3000 Da
  - Detection limits for peptides and small molecules <10 attomole/mL
  - Mass error <5 ppm RMS with automatic internal calibration

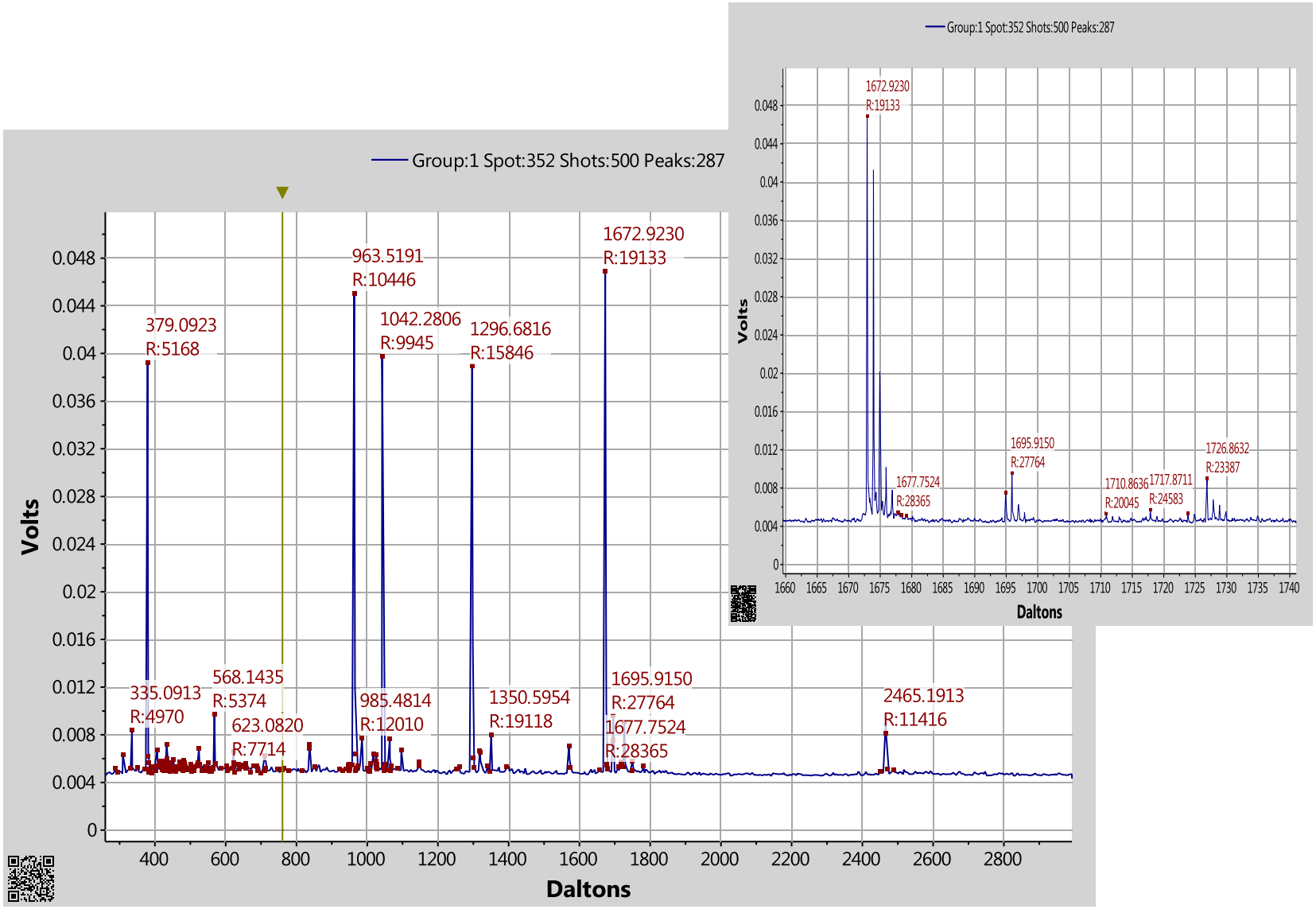


Peptide standards 1fM/μL CHCA 3000 shots from single spot
Calibration peak at 1042.285 is offscale

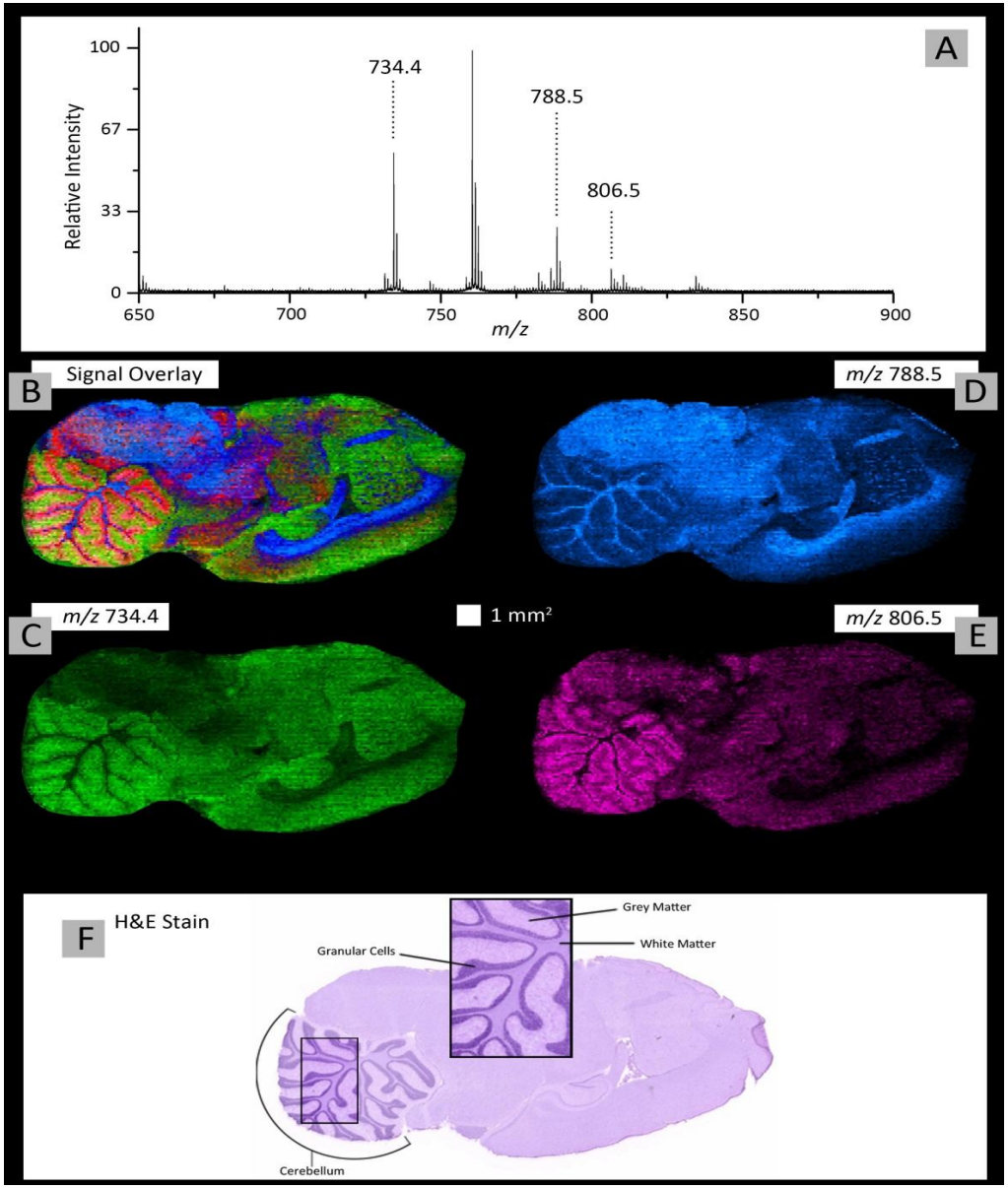
Resolving power >20,000 at focus mass
>10,000 over range 800-3000 Da



Peptide standards 100 fM/µL CHCA 500 shots



Peptide standards 100 fM/μL CHCA 500 shots, higher intensity



Lipids using prototype reflector

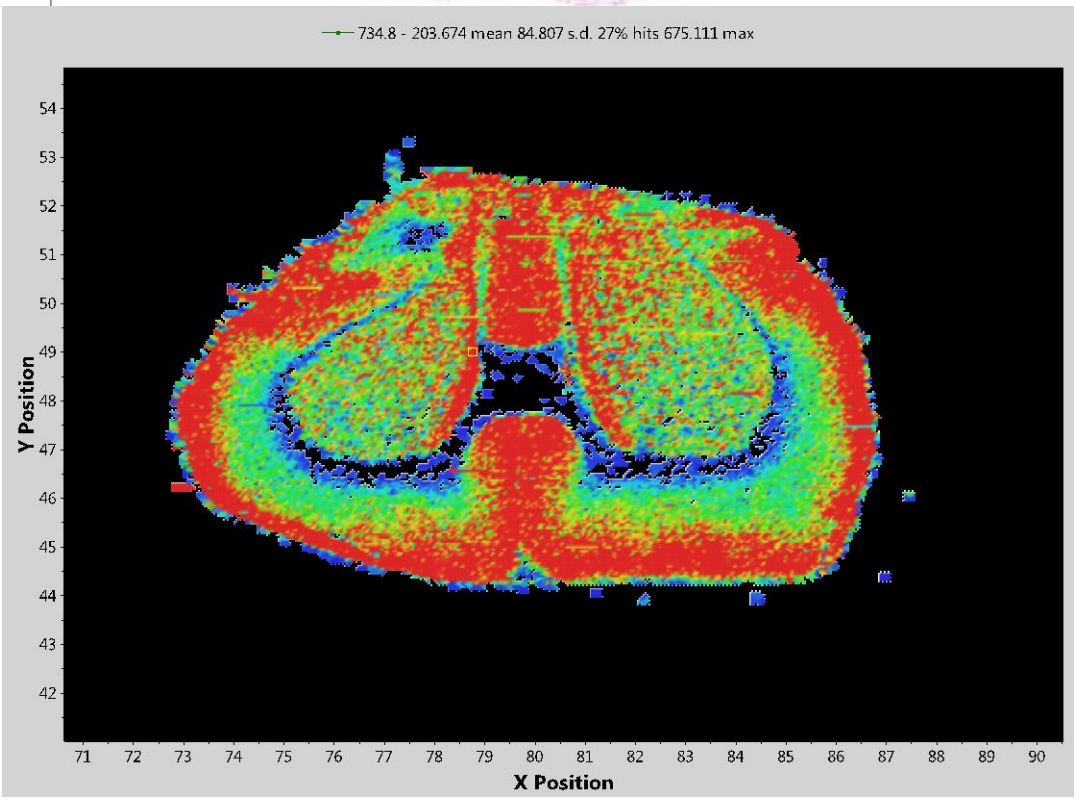
Figure 1: **A 100 µm spatial resolution lipid ion image of a sagittal rat brain tissue section using typewriter continuous laser raster sampling acquired in 10 minutes**. A representative spectrum is shown in A. The ion image overlay (B) of signal from *m/z* 734.4 (C), *m/z* 788.5 (D) and *m/z* 806.5 (E) highlights the differentiation of the spatial distributions for the selected ions. These results correlate to the H&E stained serial tissue section highlighting structural difference between grey matter, white matter and granular cells in the cerebellum (F). Important instrumental parameters:

3 kHz laser repetition rate, 5 mm/s sample stage velocity, and 60 laser shots/spectrum hardware average.

J. M. Spraggins and Richard Caprioli, Mass Spectrometry Research Center and Department of Biochemistry, Chemistry, Pharmacology, and Medicine, Vanderbilt University Medical Center

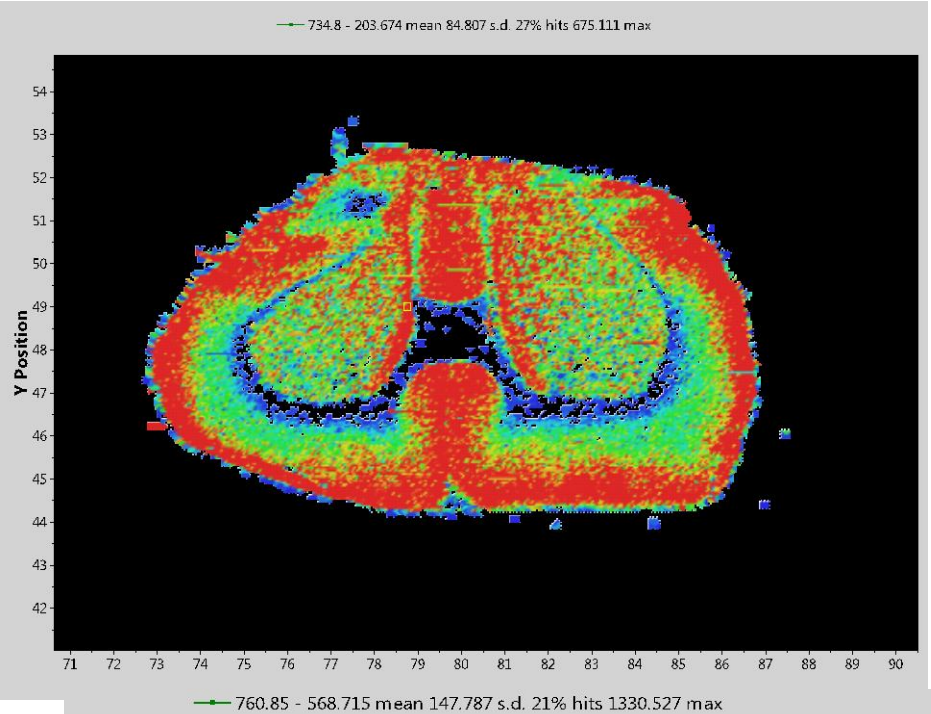
JASMS 2011

734.6

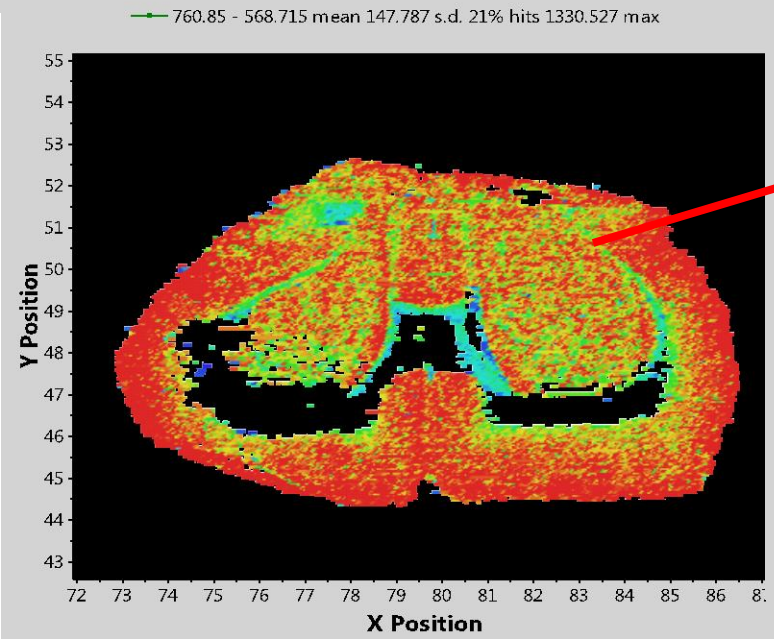


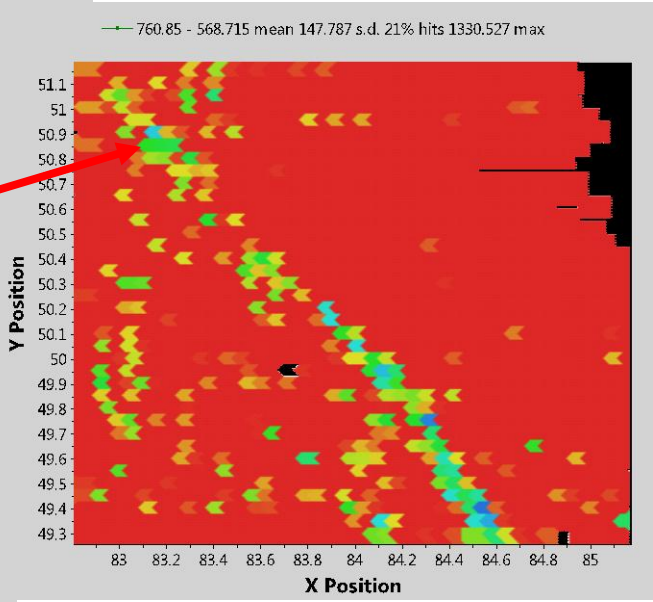
SimulTOF 200 in Reflector Mode
110,159 spectra
50x50 μm pixels
50 spectra/s at
5 kHz, 2.5 mm/s
100 shots/pixel
45 minutes total

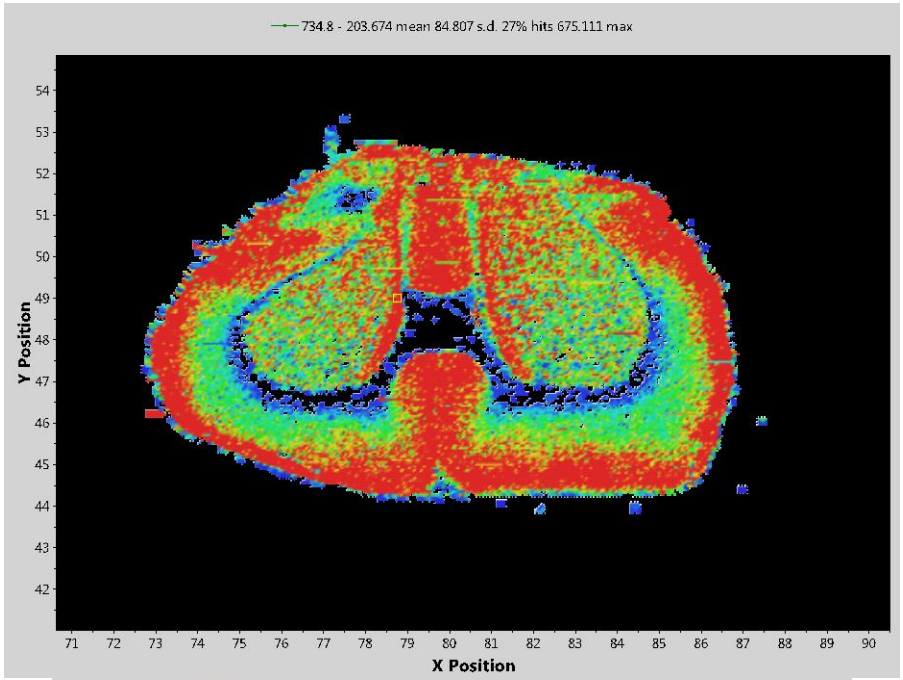
734.6


734.8 - 203.674 mean 84.807 s.d. 27% hits 675.111 max
Y Position
54
53
52
51
50
49
48
47
46
45
44
43
42
71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90

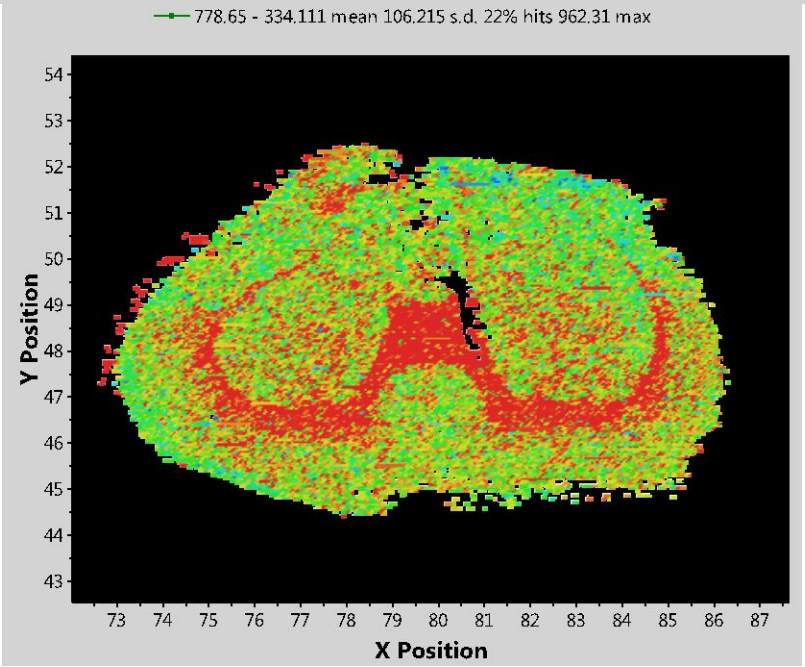
760.6


760.85 - 568.715 mean 147.787 s.d. 21% hits 1330.527 max
Y Position
55
54
53
52
51
50
49
48
47
46
45
44
43
72 73 74 75 76 77 78 79 80 81 82 83 84 85 86
X Position


760.85 - 568.715 mean 147.787 s.d. 21% hits 1330.527 max
Y Position
51.1
51
50.9
50.8
50.7
50.6
50.5
50.4
50.3
50.2
50.1
50
49.9
49.8
49.7
49.6
49.5
49.4
49.3
83 83.2 83.4 83.6 83.8 84 84.2 84.4 84.6 84.8 85
X Position


734.8 - 203.674 mean 84.807 s.d. 27% hits 675.111 max
Y Position
X Position

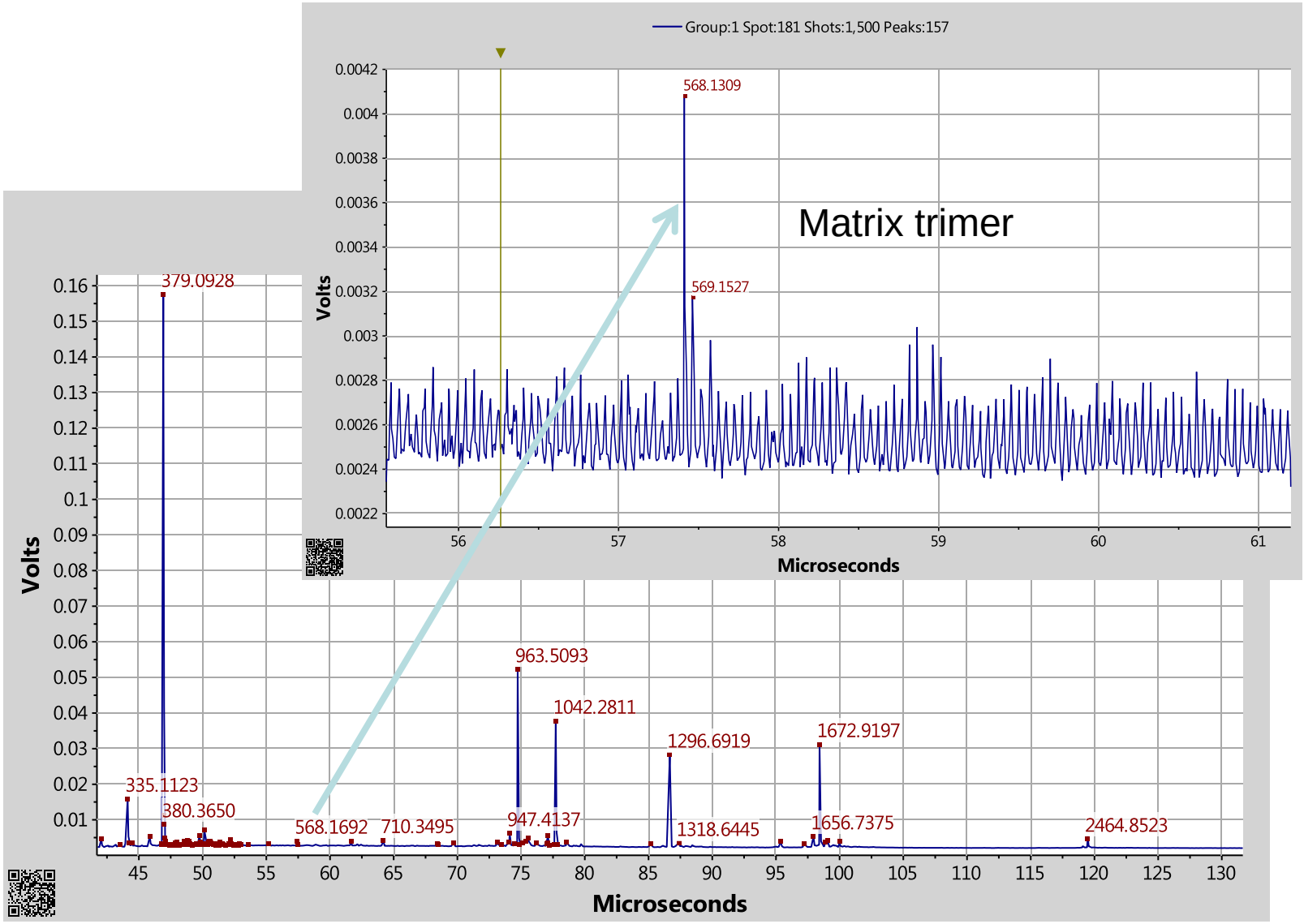
734.6

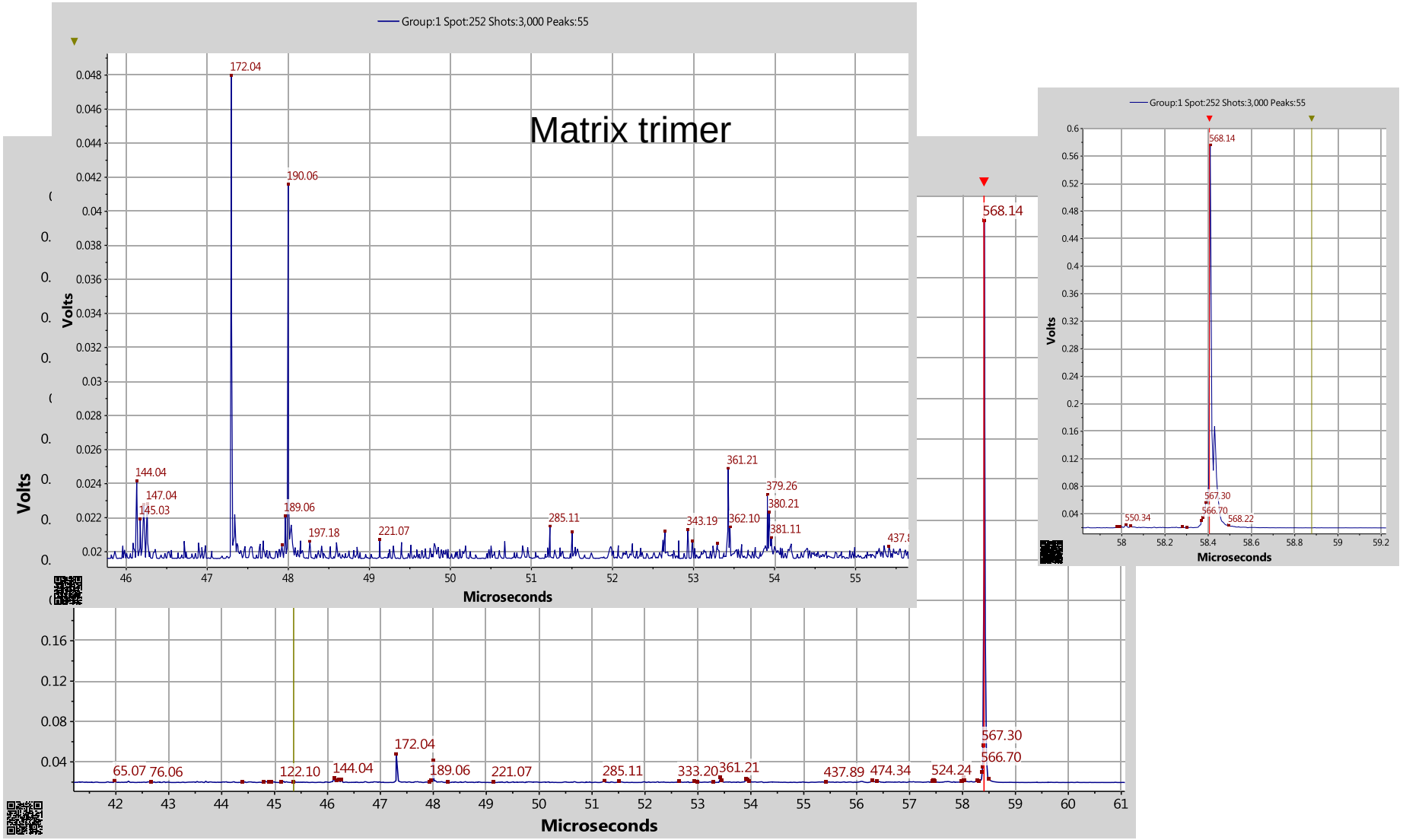

778.65 - 334.111 mean 106.215 s.d. 22% hits 962.31 max
Y Position
X Position

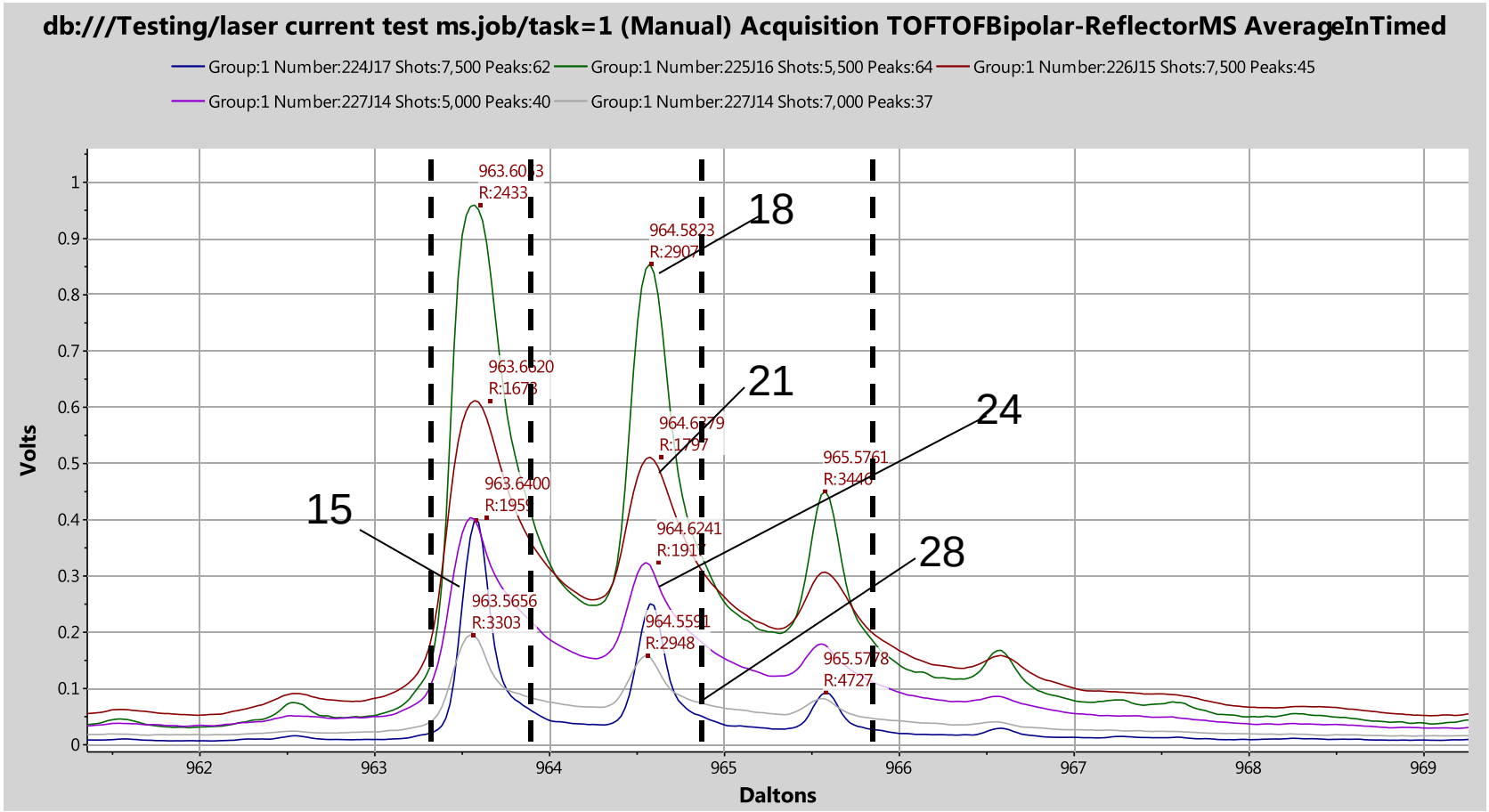
778.6

Specifications for SimulTOF 300 MS-MS

- High laser rate (5 kHz),multiplexed MS-MS (10x,) and high acquisition rate (up to 50 spectra/s) makes tissue imaging and high throughput LC-MS-MS practical
- Proprietary ion optics and high laser rate provide sensitivity and dynamic range limited only by chemical noise
- High resolution precursor selection (>1000)
- Provides both PSD and CID fragment spectra with high sensitivity, high resolving power, and excellent mass accuracy
- Performance substantially exceeds that of all existing MALDI MS-MS instruments
- Efficient structure determination for molecules detected by MALDI-MS

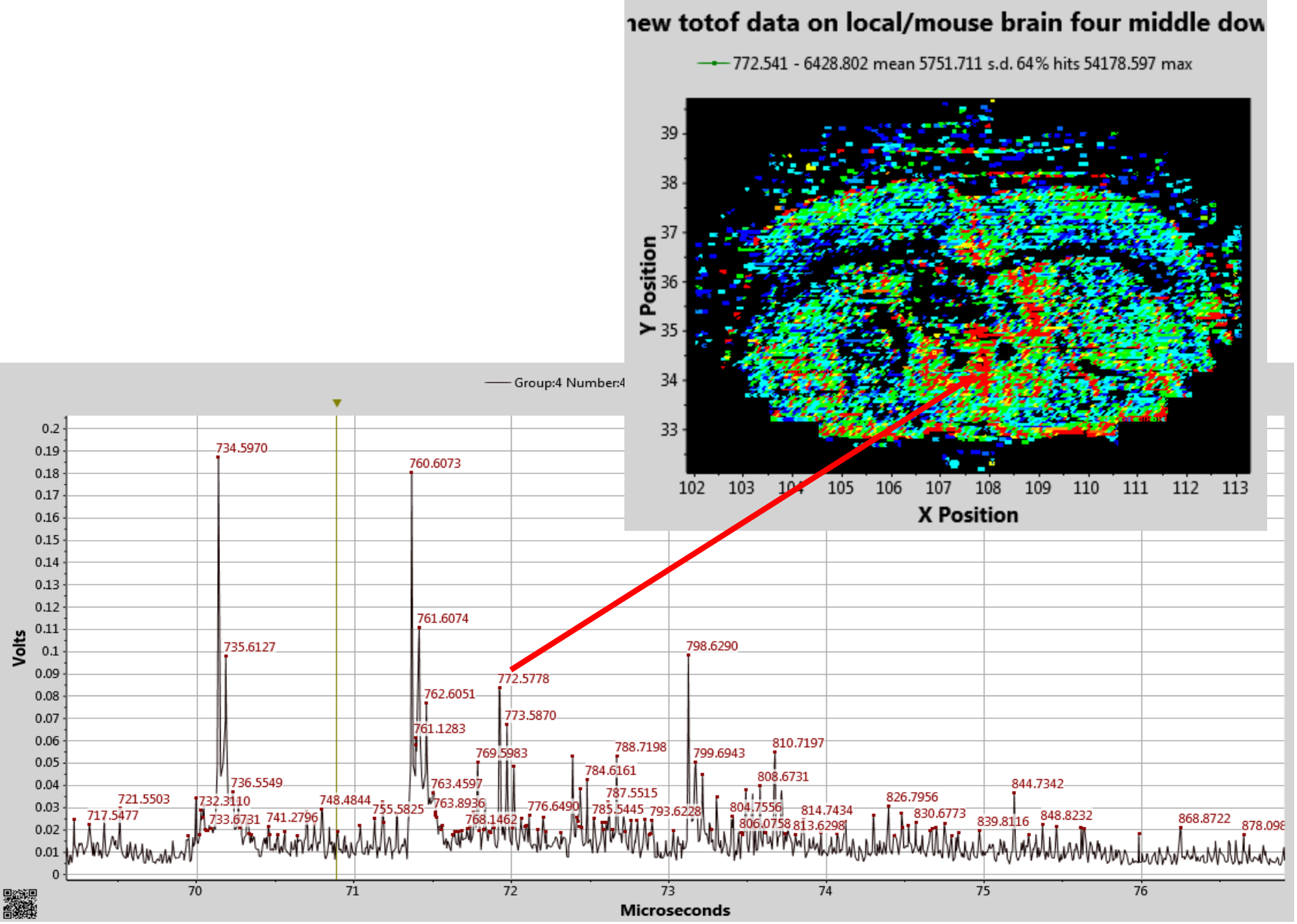

Group:1 Spot:181 Shots:1,500 Peaks:157
Matrix trimer
568.1309
569.1527
Volts
Microseconds
379.0928
963.5093
1042.2811
1296.6919
1672.9197
335.1123
380.3650
568.1692
710.3495
947.4137
1318.6445
1656.7375
2464.8523


Group:1 Spot:252 Shots:3,000 Peaks:55
Matrix trimer
Volts
Microseconds
172.04
190.06
144.04
147.04
145.03
189.06
197.18
221.07
285.11
343.19
361.21
362.10
379.26
380.21
381.11
437.8
568.14
567.30
566.70
65.07
76.06
122.10
144.04
172.04
189.06
221.07
285.11
333.20
361.21
437.89
474.34
524.24
550.34
568.22



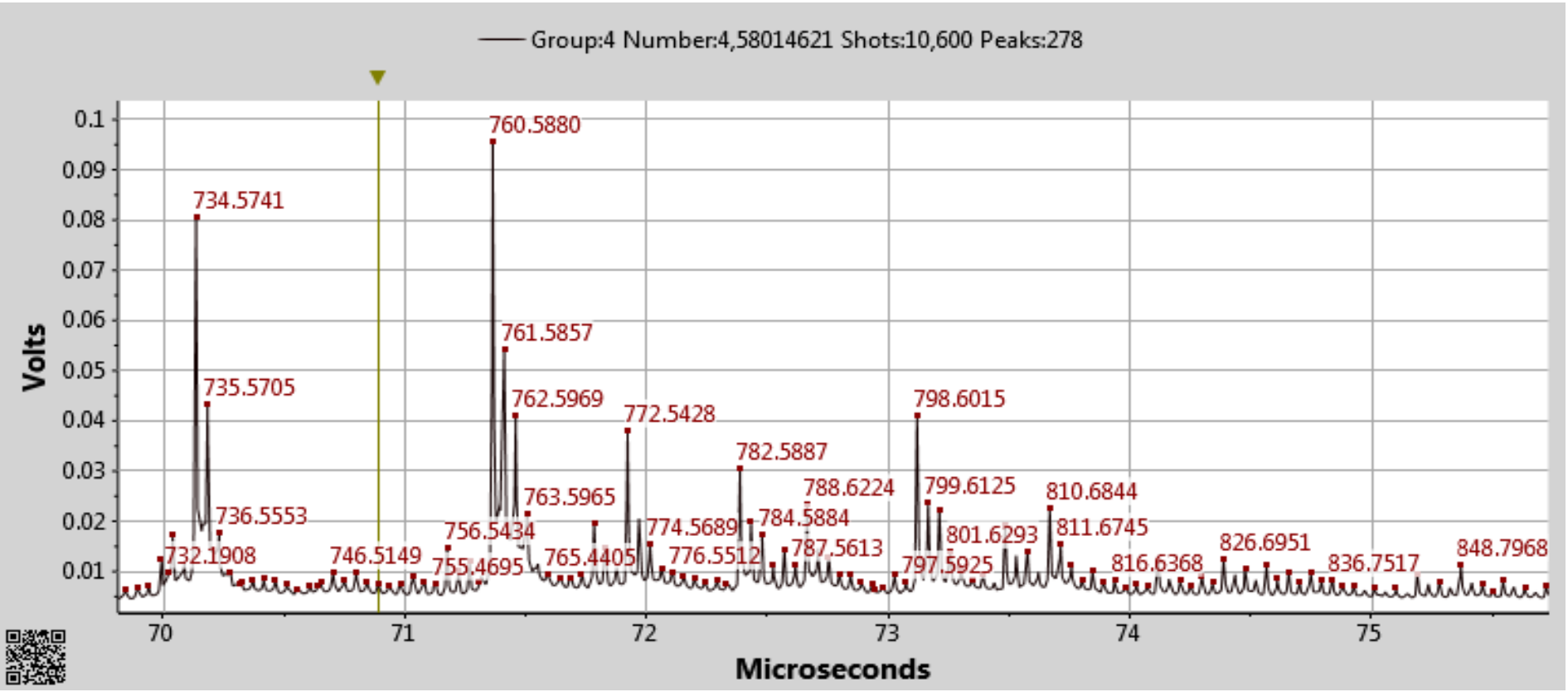
parameter is fluence in μJ

Imaging of lipids with positive ions

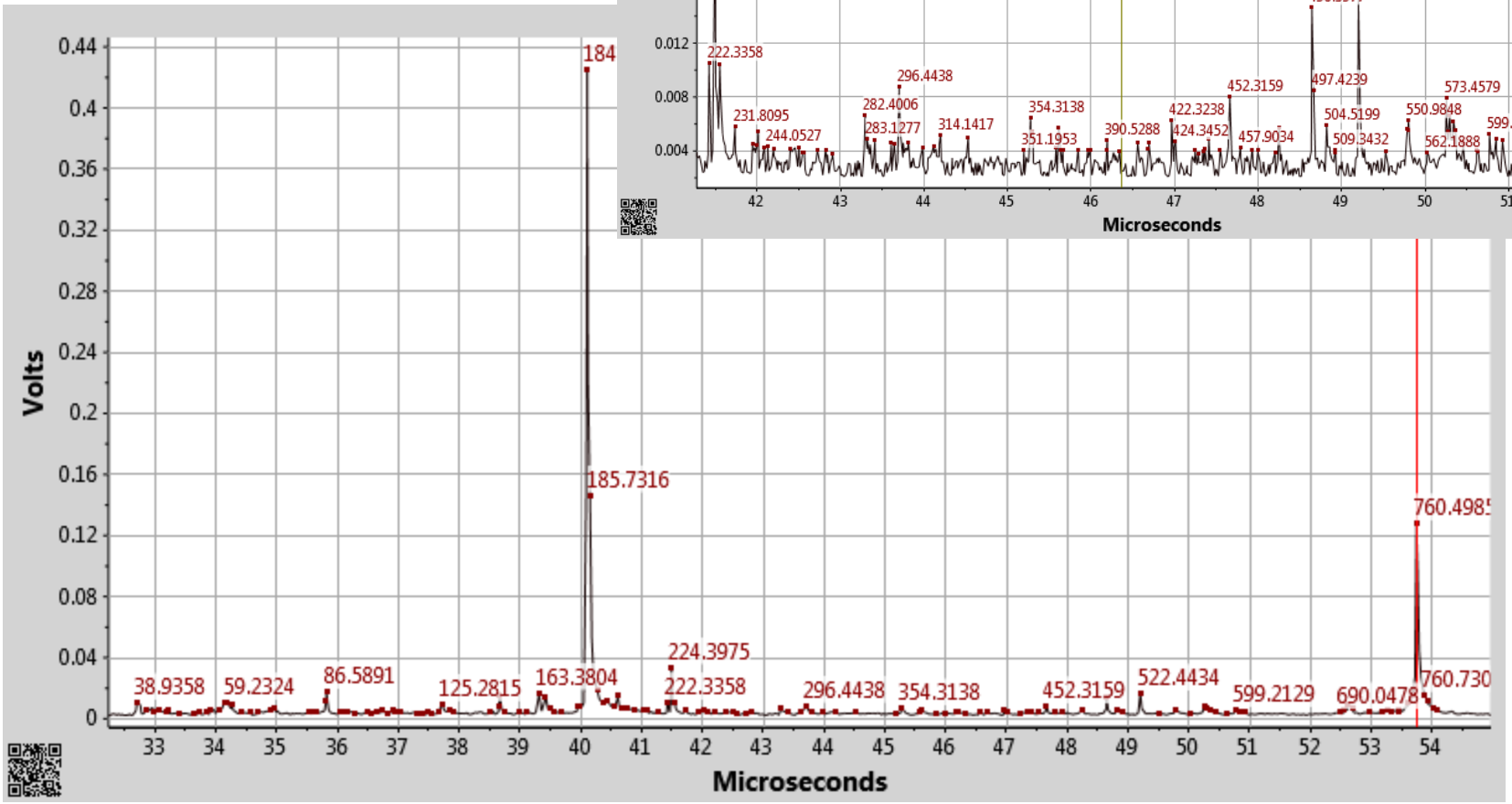


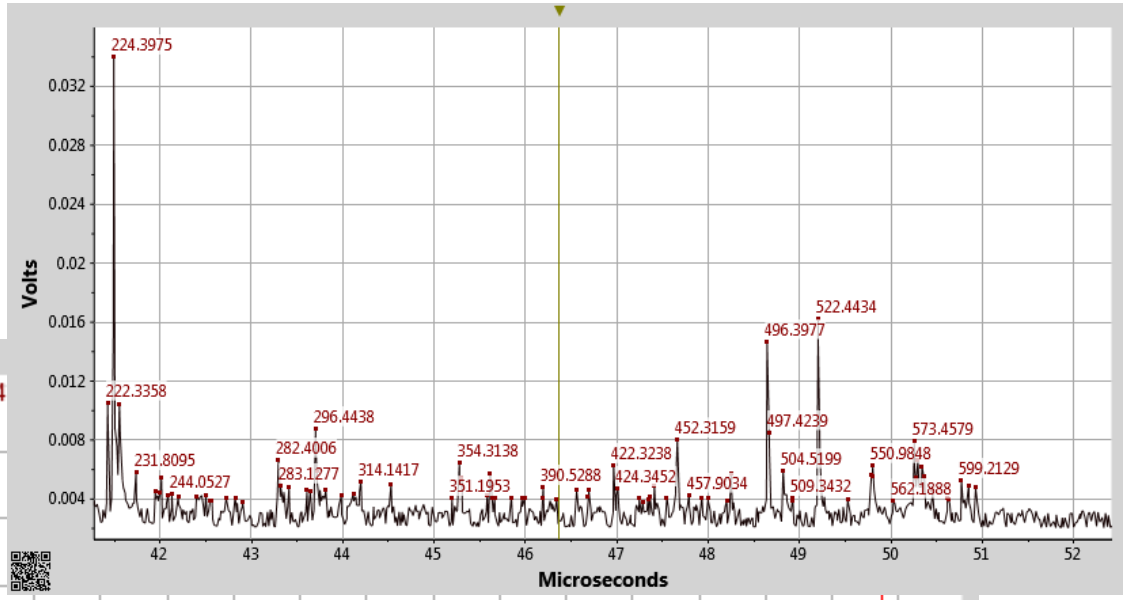
100 shot spectrum from single 50x50 μm pixel at 5 kHz

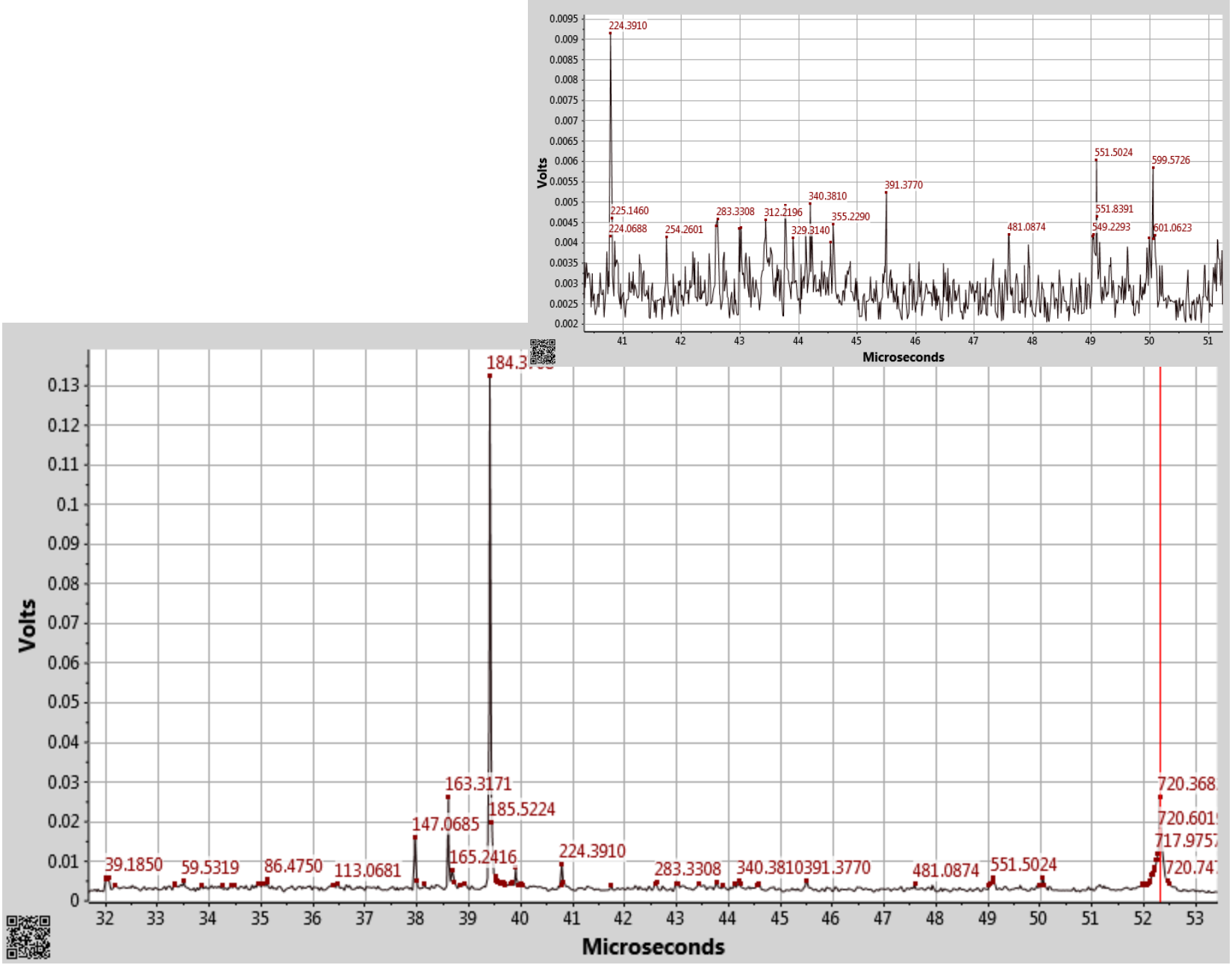
10,600 shots @ 5 kHz



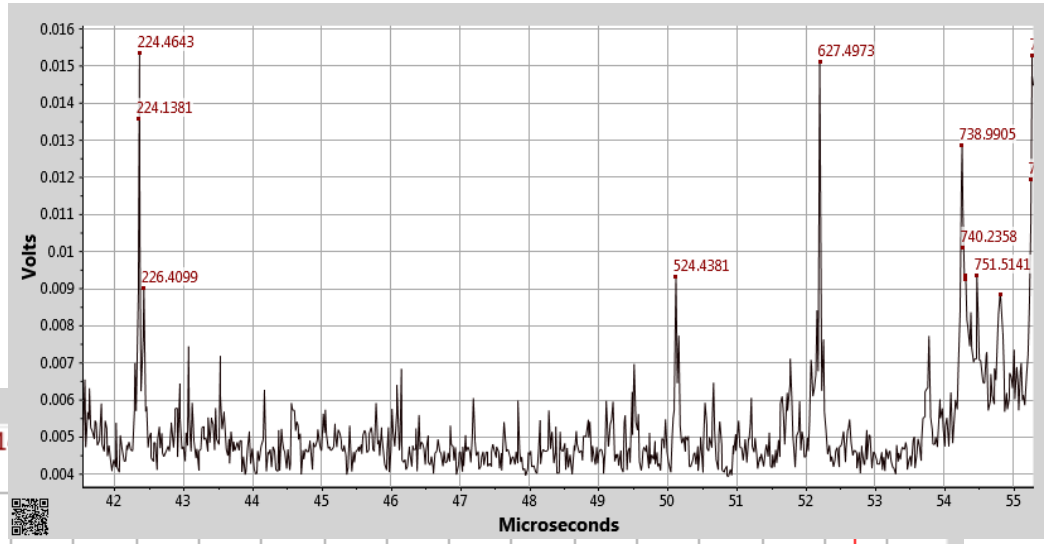
MS-MS
Diacyl GPCho 34a:1

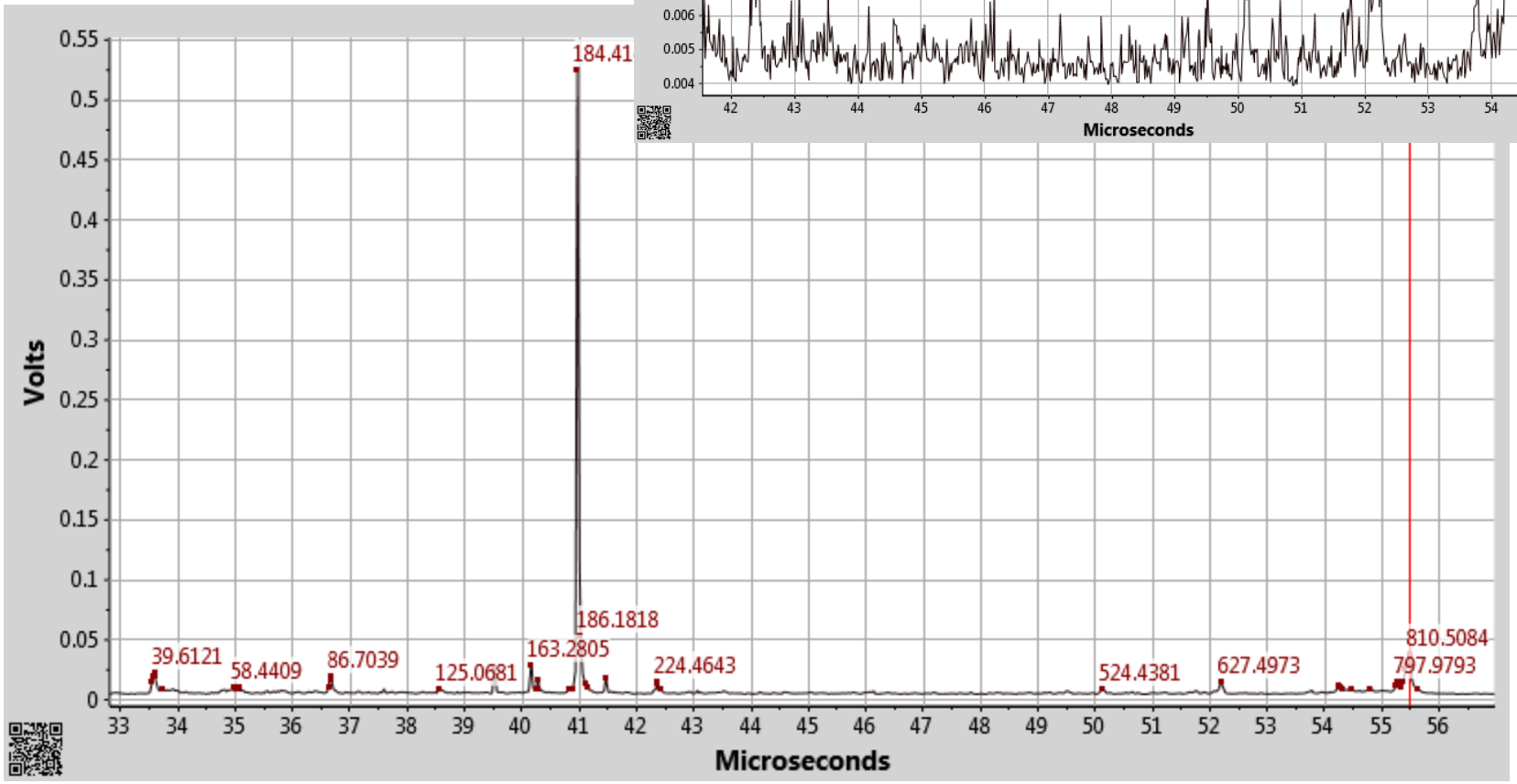




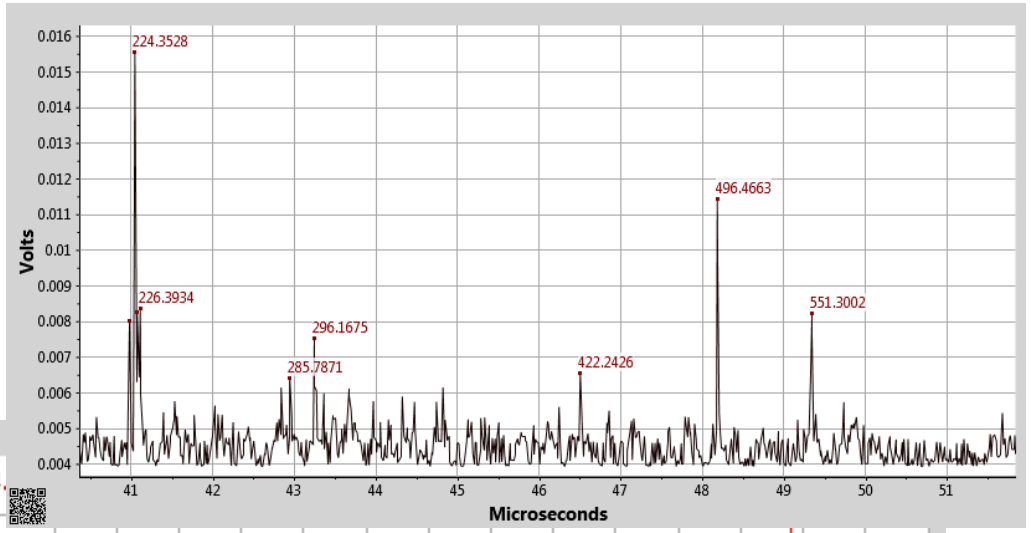

Volts
Microseconds
224.3910
225.1460
224.0688
254.2601
283.3308
312.2196
329.3140
340.3810
355.2290
391.3770
481.0874
551.5024
551.8391
549.2293
599.5726
601.0623
184.3708
163.3171
185.5224
147.0685
165.2416
39.1850
59.5319
86.4750
113.0681
224.3910
283.3308
340.3810
391.3770
481.0874
551.5024
720.368
720.601
717.9757
720.74

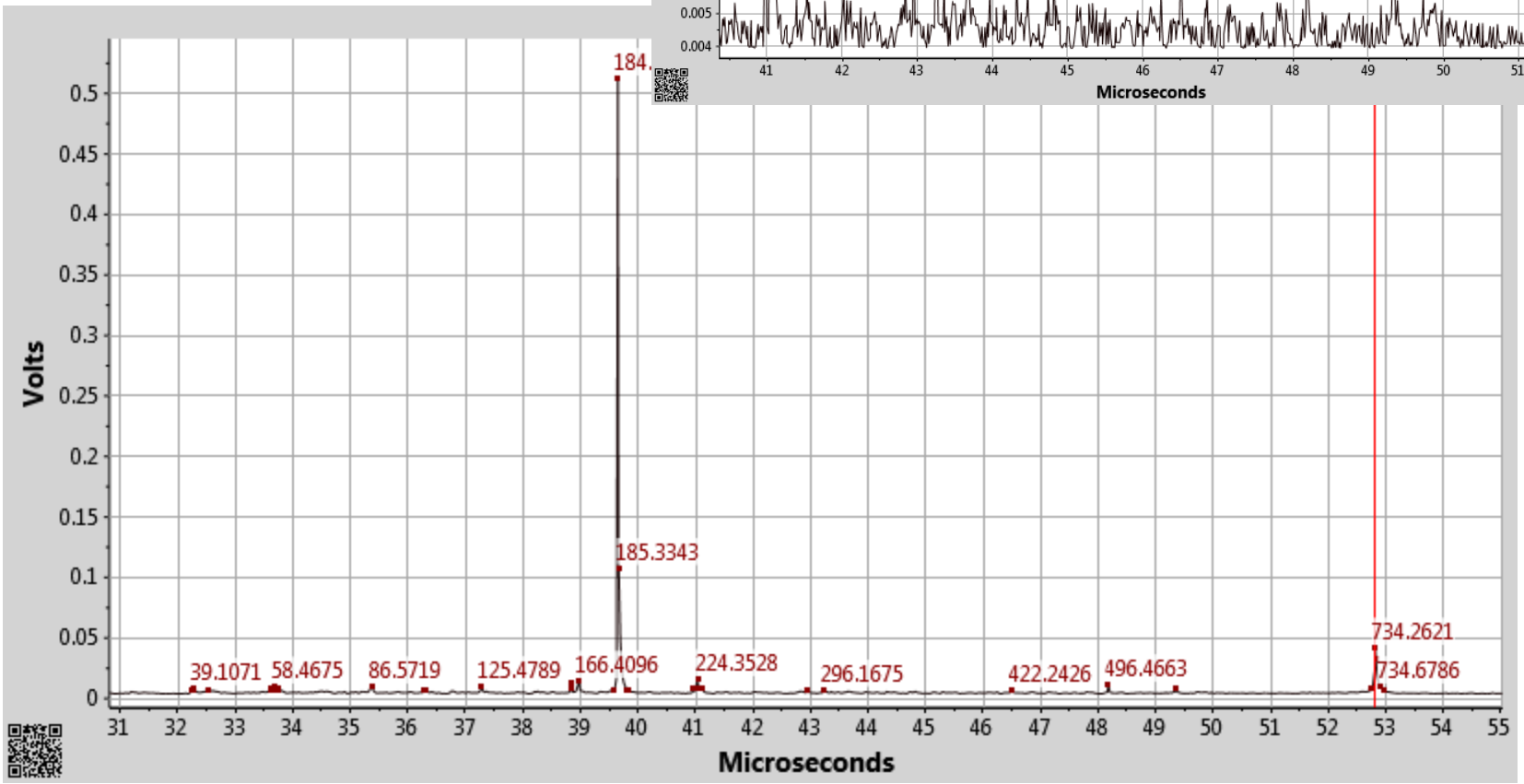
MS-MS
Diacyl GPCho 38a:4





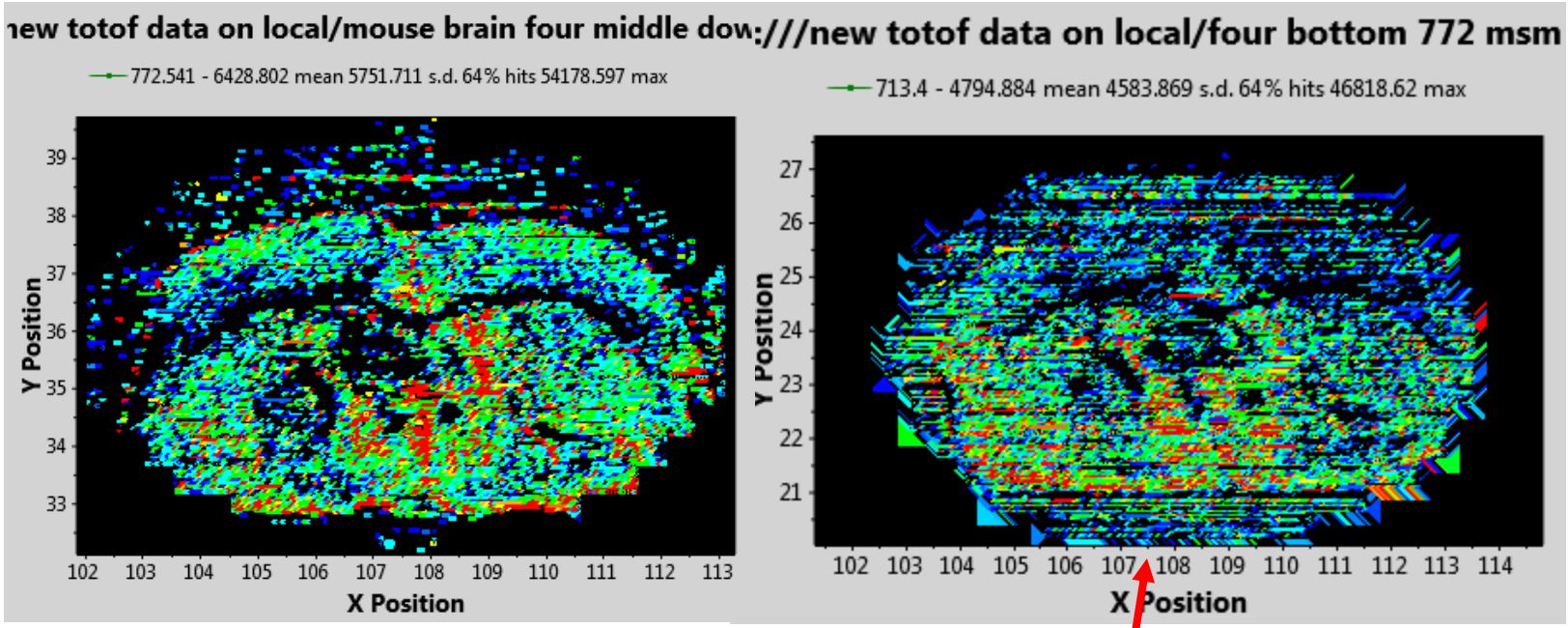
MS-MS
Diacyl GPCho 32a:0

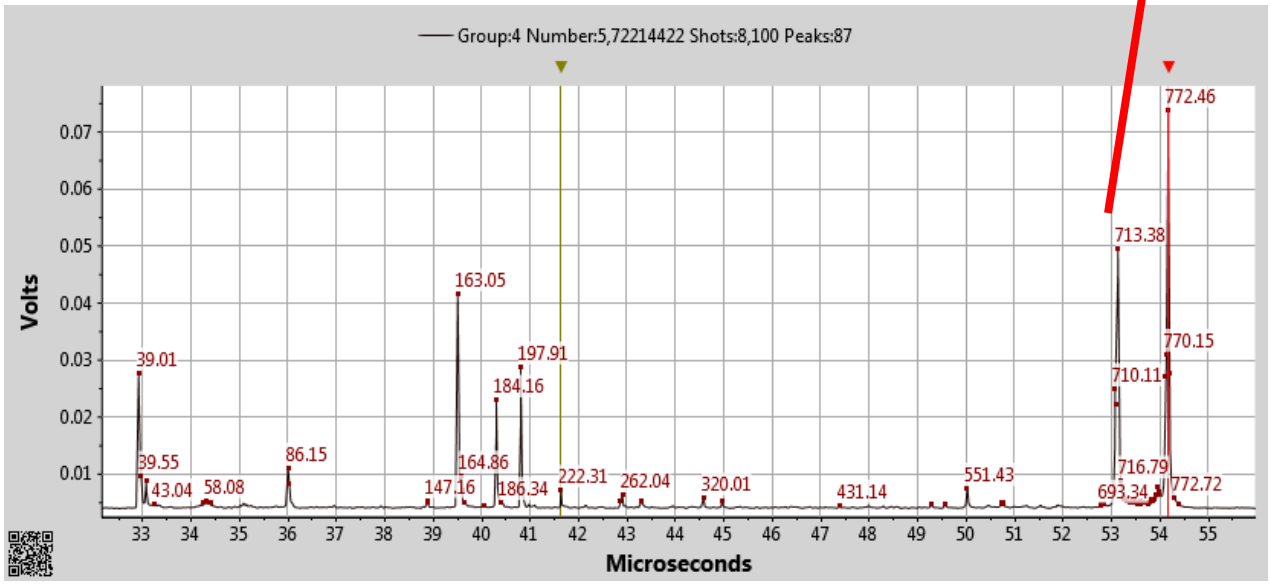




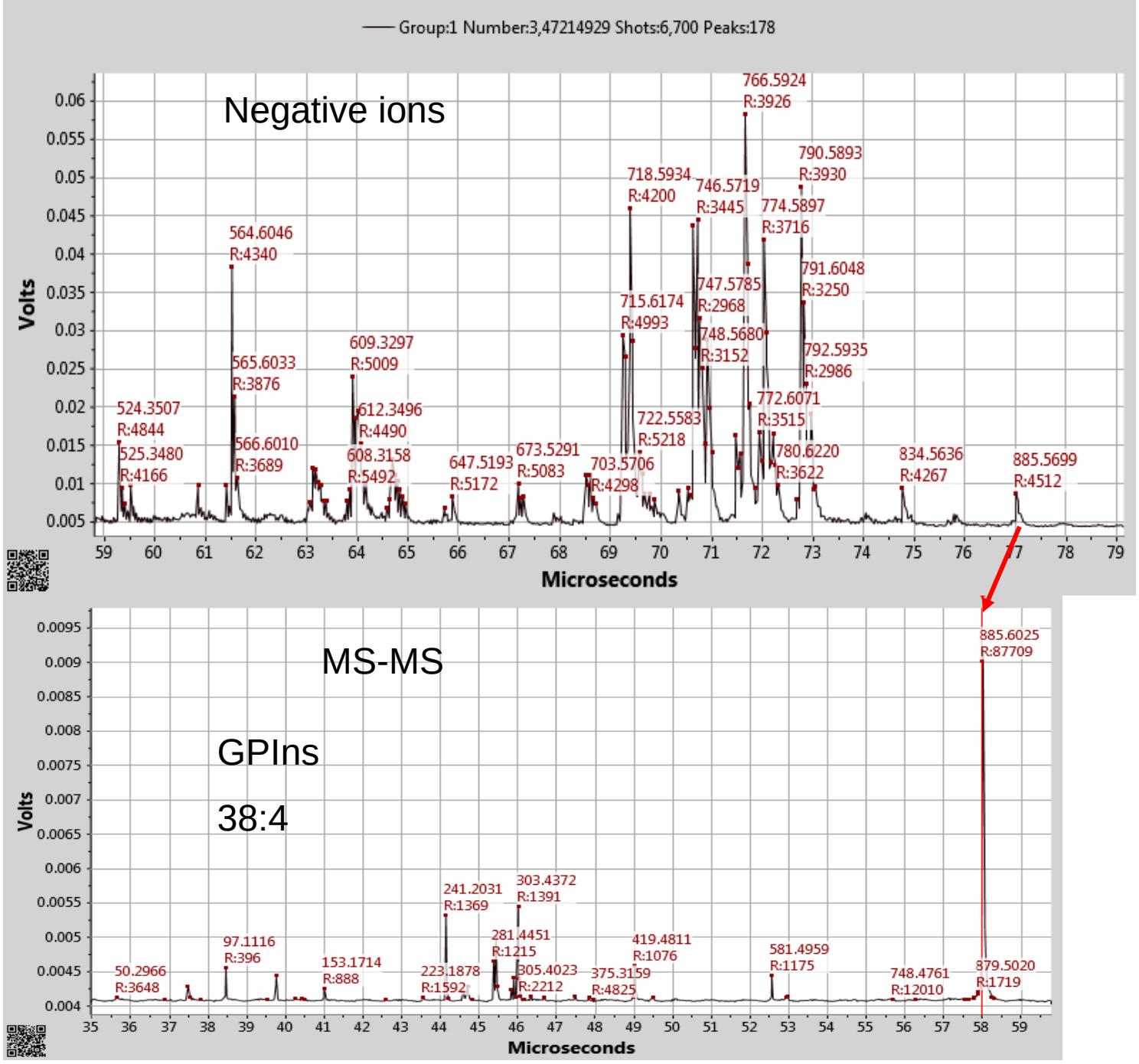
MS-MS

Diacyl GPCho 32a:0+K^+

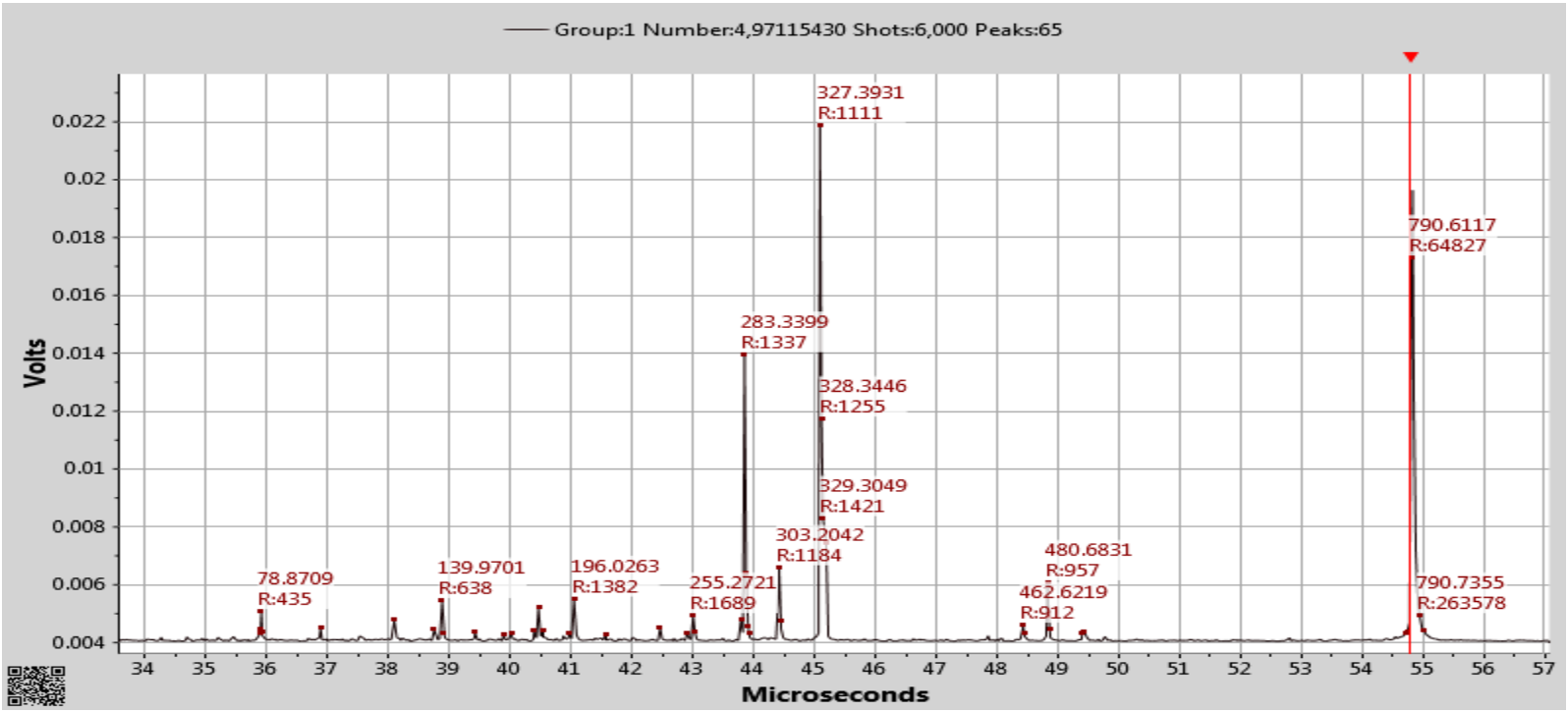


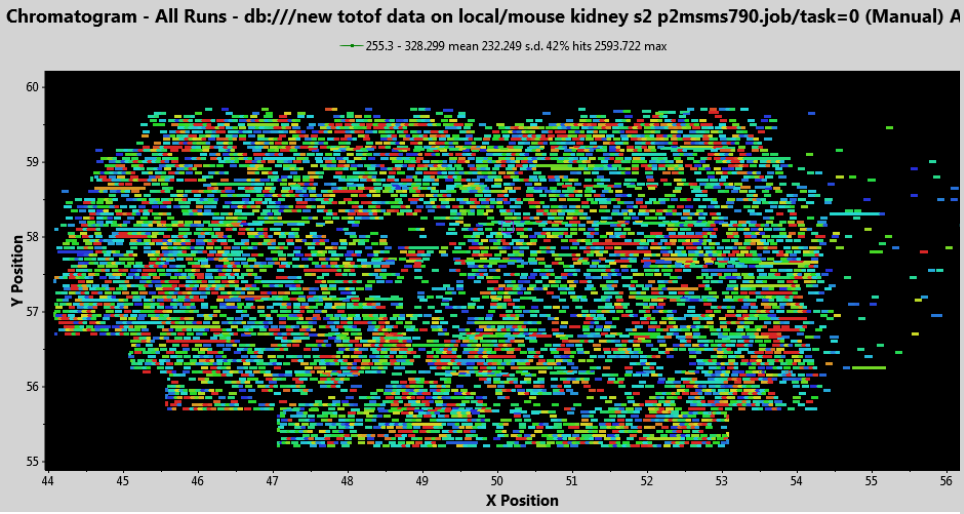


Imaging of lipids with negative ions

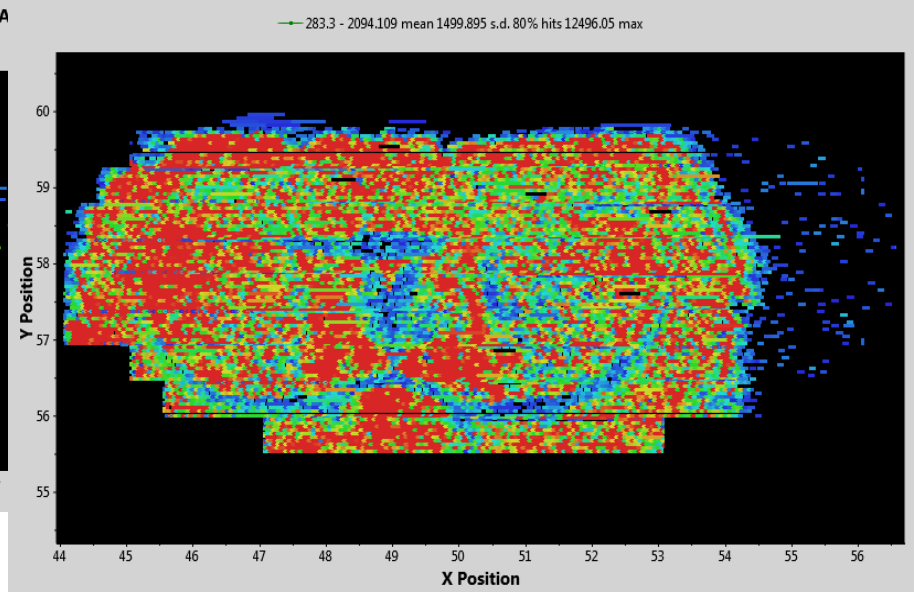

Group:1 Number:3,47214929 Shots:6,700 Peaks:178
Negative ions
Volts
Microseconds
766.5924 R:3926
790.5893 R:3930
718.5934 R:4200
746.5719 R:3445
774.5897 R:3716
564.6046 R:4340
791.6048 R:3250
747.5785 R:2968
715.6174 R:4993
748.5680 R:3152
609.3297 R:5009
565.6033 R:3876
792.5935 R:2986
772.6071 R:3515
524.3507 R:4844
612.3496 R:4490
722.5583 R:5218
566.6010 R:3689
780.6220 R:3622
525.3480 R:4166
608.3158 R:5492
673.5291 R:5083
647.5193 R:5172
703.5706 R:4298
834.5636 R:4267
885.5699 R:4512
MS-MS
GPIns
38:4
Volts
Microseconds
885.6025 R:87709
303.4372 R:1391
241.2031 R:1369
281.4451 R:1215
419.4811 R:1076
97.1116 R:396
581.4959 R:1175
153.1714 R:888
50.2966 R:3648
223.1878 R:1592
305.4023 R:2212
375.3159 R:4825
748.4761 R:12010
879.5020 R:1719

GPEtn 40a:6

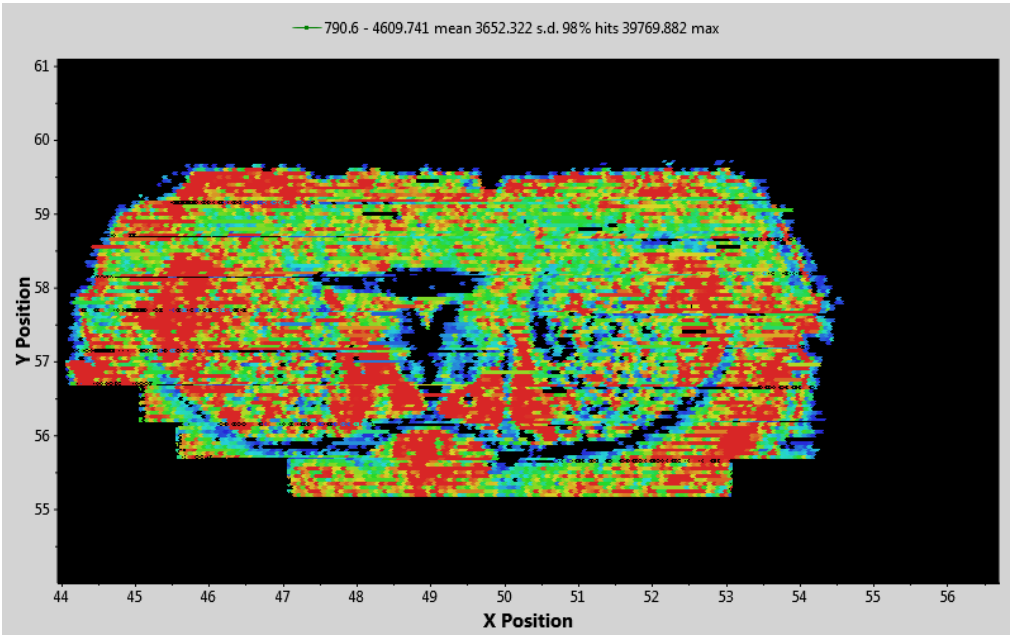

Group:1 Number:4,97115430 Shots:6,000 Peaks:65
Volts
Microseconds
0.022
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
327.3931
R:1111
790.6117
R:64827
283.3399
R:1337
328.3446
R:1255
329.3049
R:1421
303.2042
R:1184
480.6831
R:957
462.6219
R:912
78.8709
R:435
139.9701
R:638
196.0263
R:1382
255.2721
R:1689
790.7355
R:263578


Chromatogram - All Runs - db:///new totof data on local/mouse kidney s2 p2msms790.job/task=0 (Manual) A
255.3 - 328.299 mean 232.249 s.d. 42% hits 2593.722 max
Y Position
X Position

255

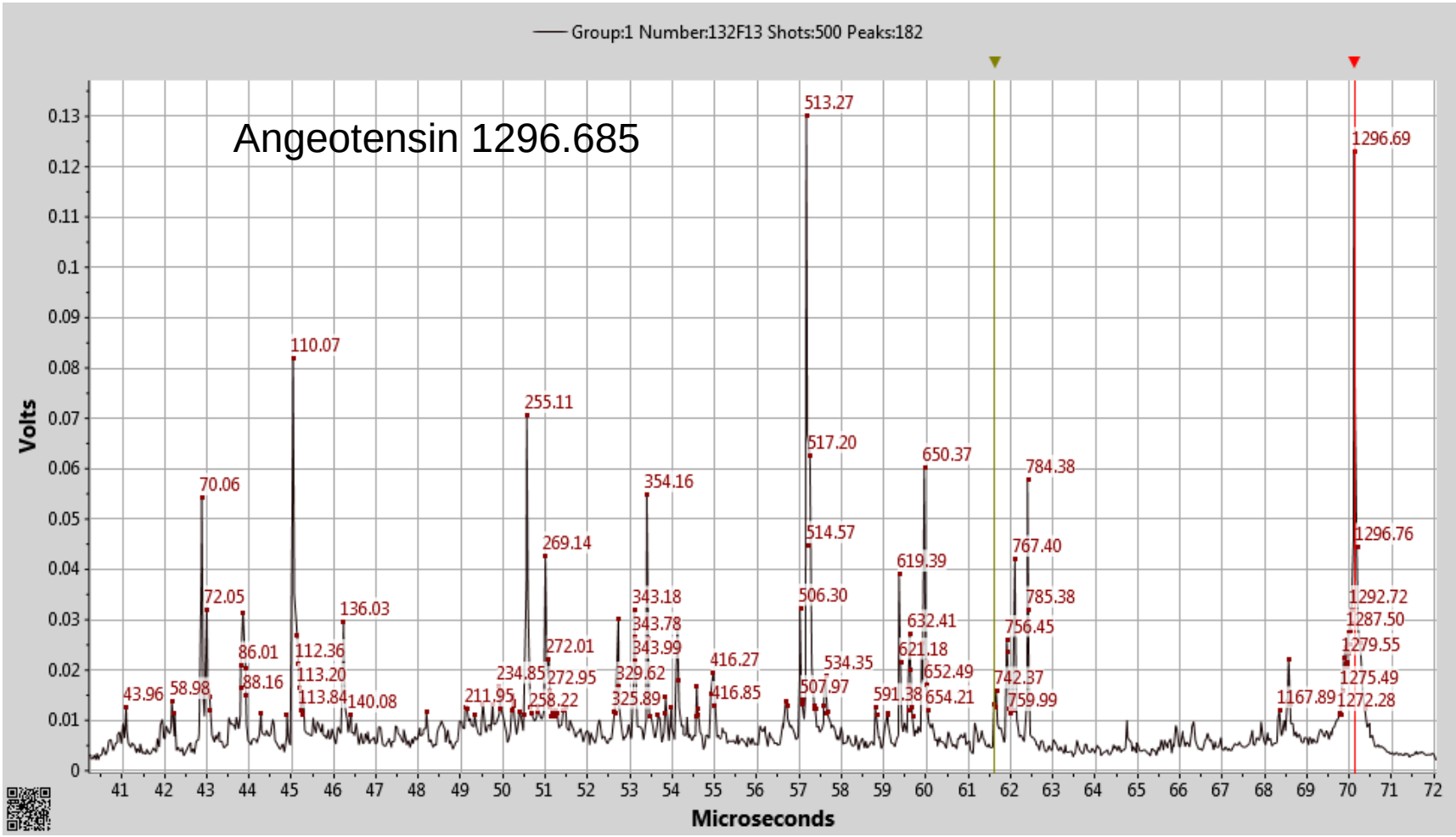

283.3 - 2094.109 mean 1499.895 s.d. 80% hits 12496.05 max
Y Position
X Position

283

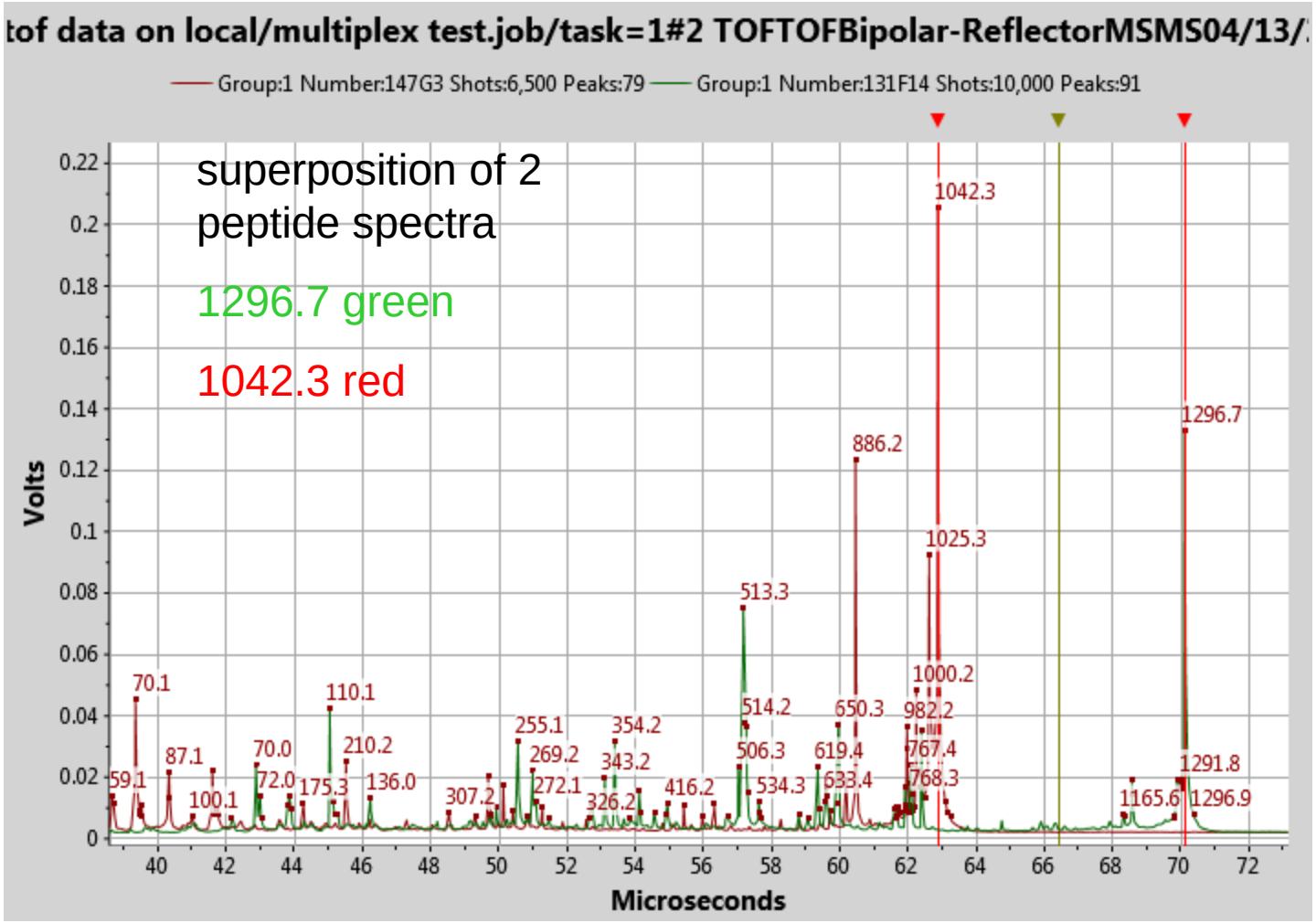

790.6 - 4609.741 mean 3652.322 s.d. 98% hits 39769.882 max
Y Position
X Position

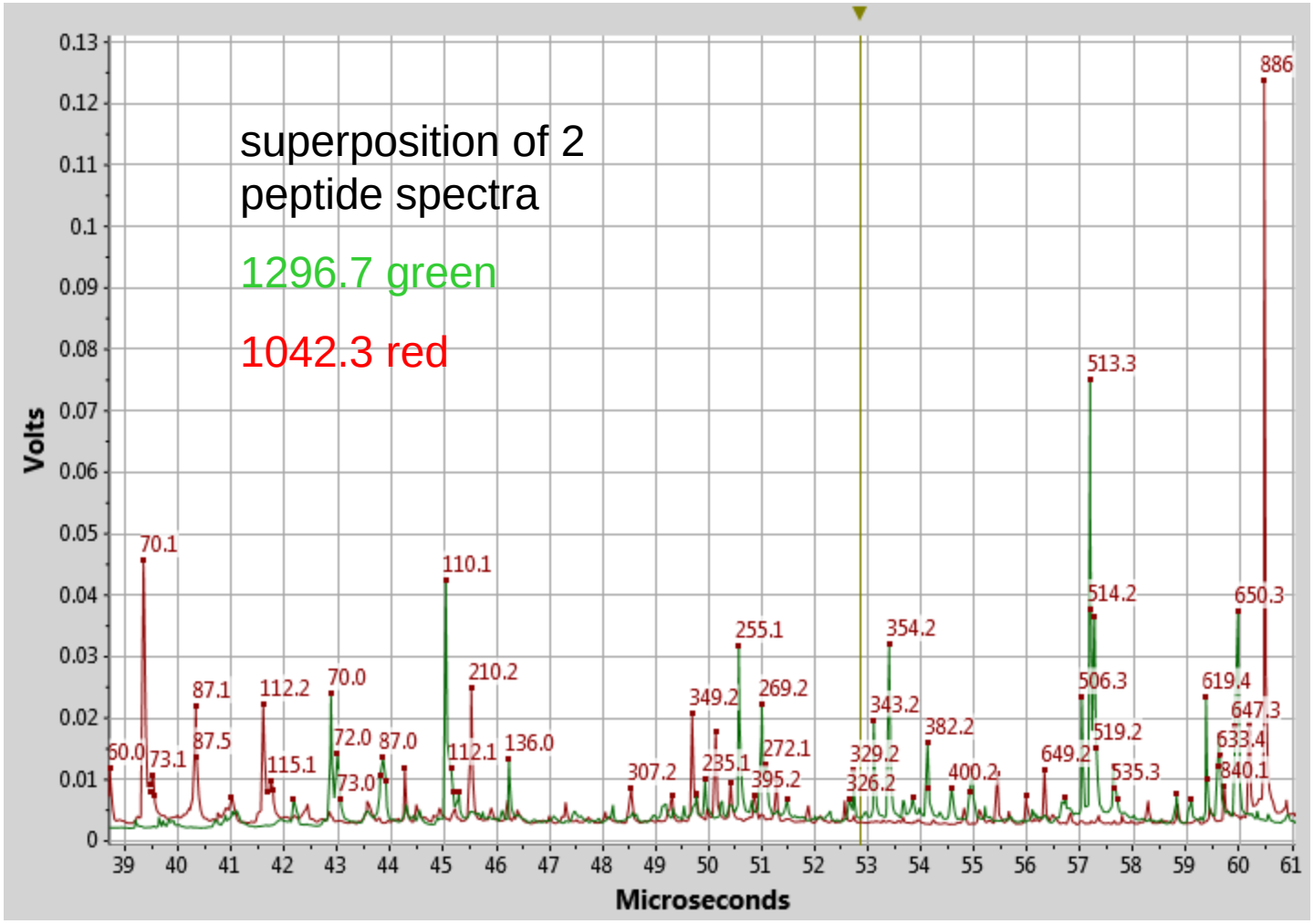
790

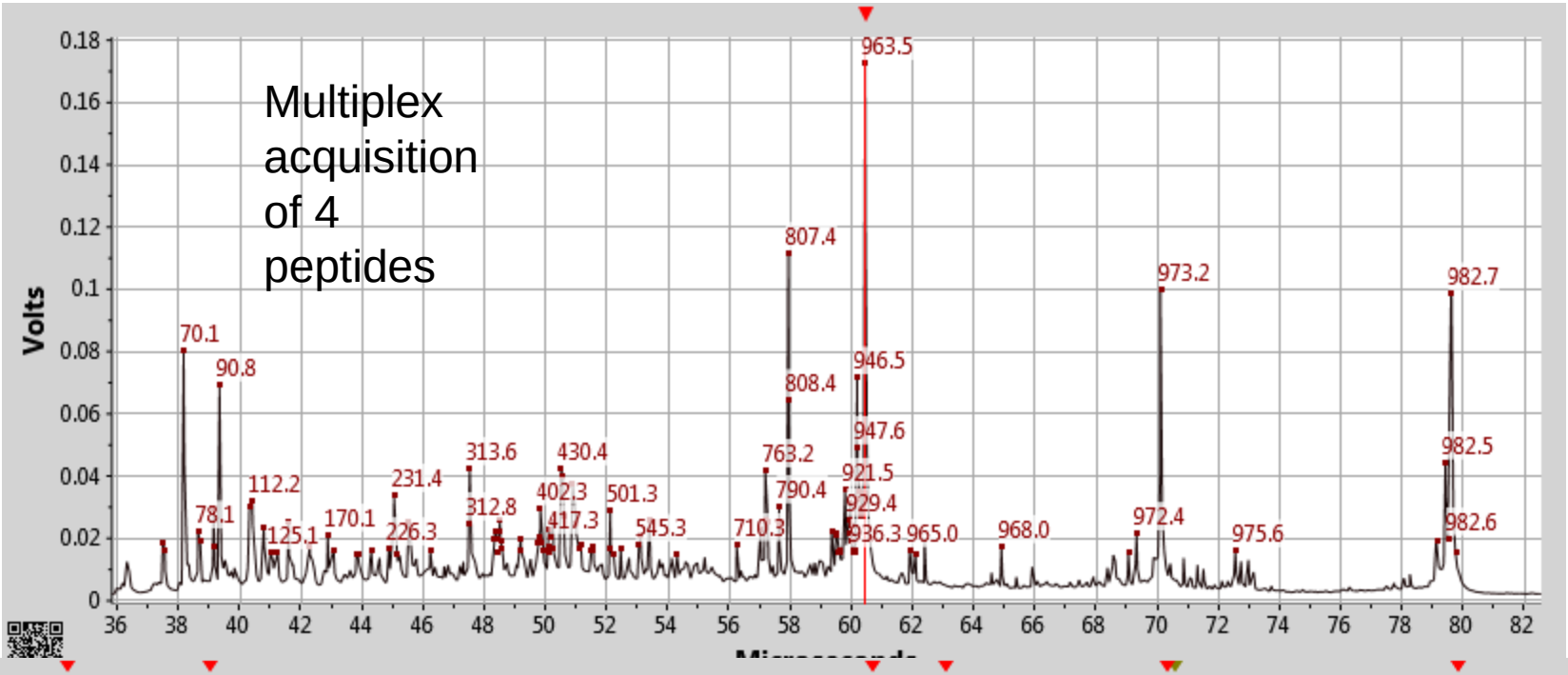
Mutiplexed MS-MS

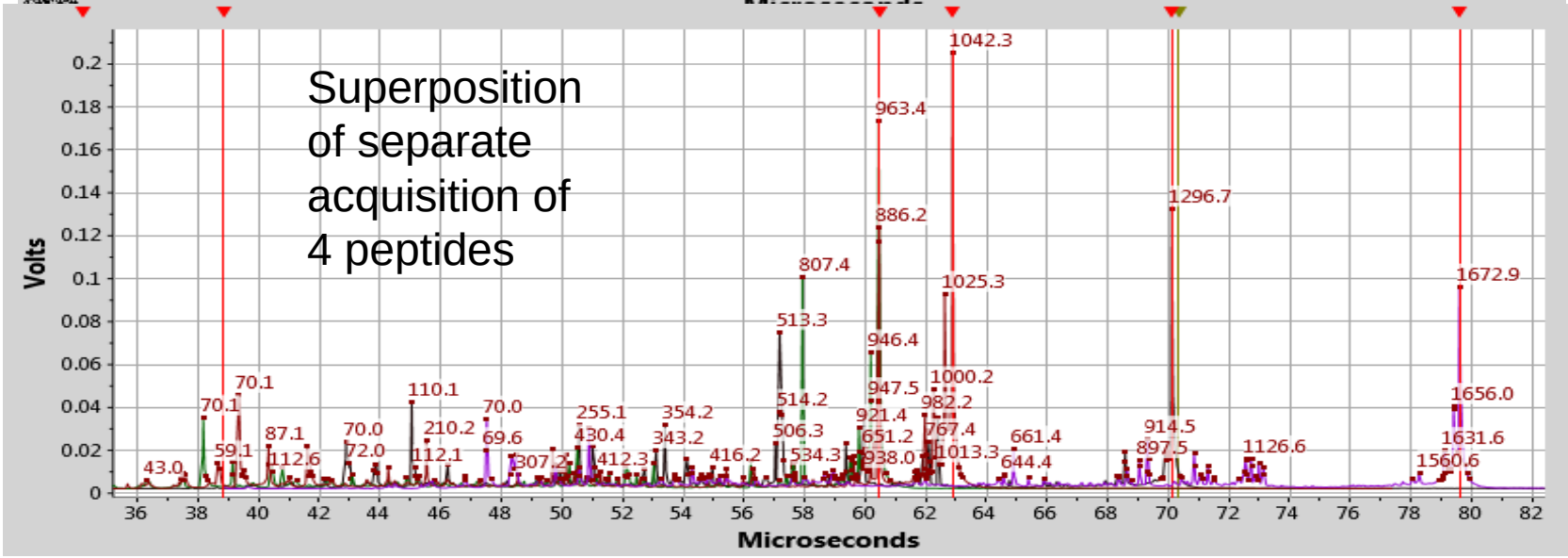


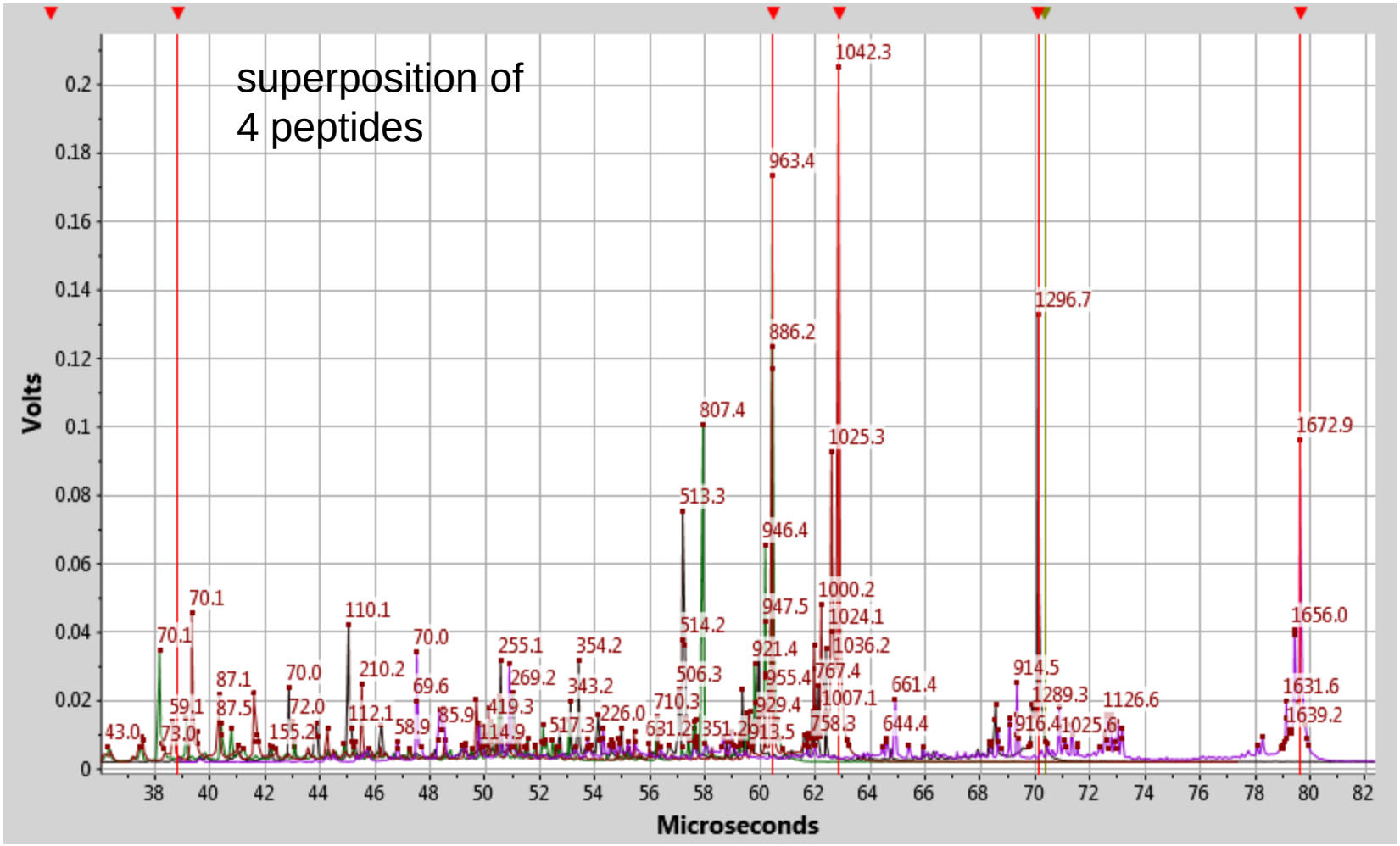
Typical ms-ms spectrum of peptide 100 fm loaded, 500 shots @ 5 kHz


tof data on local/multiplex test.job/task=1#2 TOFTOFBipolar-ReflectorMSMS04/13/
Group:1 Number:147G3 Shots:6,500 Peaks:79
Group:1 Number:131F14 Shots:10,000 Peaks:91
superposition of 2
peptide spectra
1296.7 green
1042.3 red
Volts
Microseconds
0.22
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
40
42
44
46
48
50
52
54
56
58
60
62
64
66
68
70
72
1042.3
1296.7
886.2
1025.3
513.3
1000.2
70.1
110.1
514.2
650.3
982.2
255.1
354.2
87.1
70.0
210.2
269.2
506.3
619.4
767.4
343.2
1291.8
59.1
72.0
175.3
136.0
272.1
534.3
633.4
768.3
100.1
307.2
326.2
416.2
1165.6
1296.9


superposition of 2
peptide spectra
1296.7 green
1042.3 red
Volts
Microseconds
886
513.3
70.1
110.1
514.2
650.3
255.1
354.2
210.2
87.1
112.2
70.0
349.2
269.2
506.3
619.4
343.2
647.3
382.2
519.2
633.4
87.5
72.0
87.0
112.1
136.0
272.1
329.2
649.2
60.0
73.1
115.1
73.0
307.2
235.1
395.2
326.2
400.2
535.3
840.1


Multiplex acquisition of 4 peptides
Volts
963.5
807.4
973.2
982.7
70.1
90.8
946.5
808.4
947.6
982.5
313.6
430.4
763.2
921.5
112.2
231.4
402.3
501.3
790.4
929.4
78.1
170.1
312.8
417.3
545.3
710.3
936.3
965.0
968.0
972.4
975.6
982.6
125.1
226.3


Superposition of separate acquisition of 4 peptides
Volts
Microseconds
1042.3
963.4
1296.7
886.2
807.4
1025.3
1672.9
513.3
946.4
1000.2
947.5
982.2
1656.0
70.1
110.1
70.0
514.2
921.4
70.1
255.1
354.2
87.1
210.2
69.6
506.3
651.2
767.4
914.5
1631.6
70.0
430.4
343.2
661.4
59.1
72.0
112.1
307.2
412.3
416.2
534.3
938.0
1013.3
644.4
897.5
1126.6
1560.6
43.0
112.6


superposition of
4 peptides
Volts
Microseconds
1042.3
963.4
1296.7
886.2
807.4
1025.3
1672.9
513.3
946.4
1000.2
947.5
1024.1
1656.0
514.2
1036.2
921.4
767.4
914.5
506.3
955.4
661.4
1631.6
1007.1
1289.3
1126.6
929.4
1639.2
710.3
758.3
644.4
916.4
1025.6
913.5
631.2
351.2
226.0
517.3
343.2
354.2
255.1
269.2
419.3
114.9
85.9
69.6
70.0
110.1
210.2
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87.5
59.1
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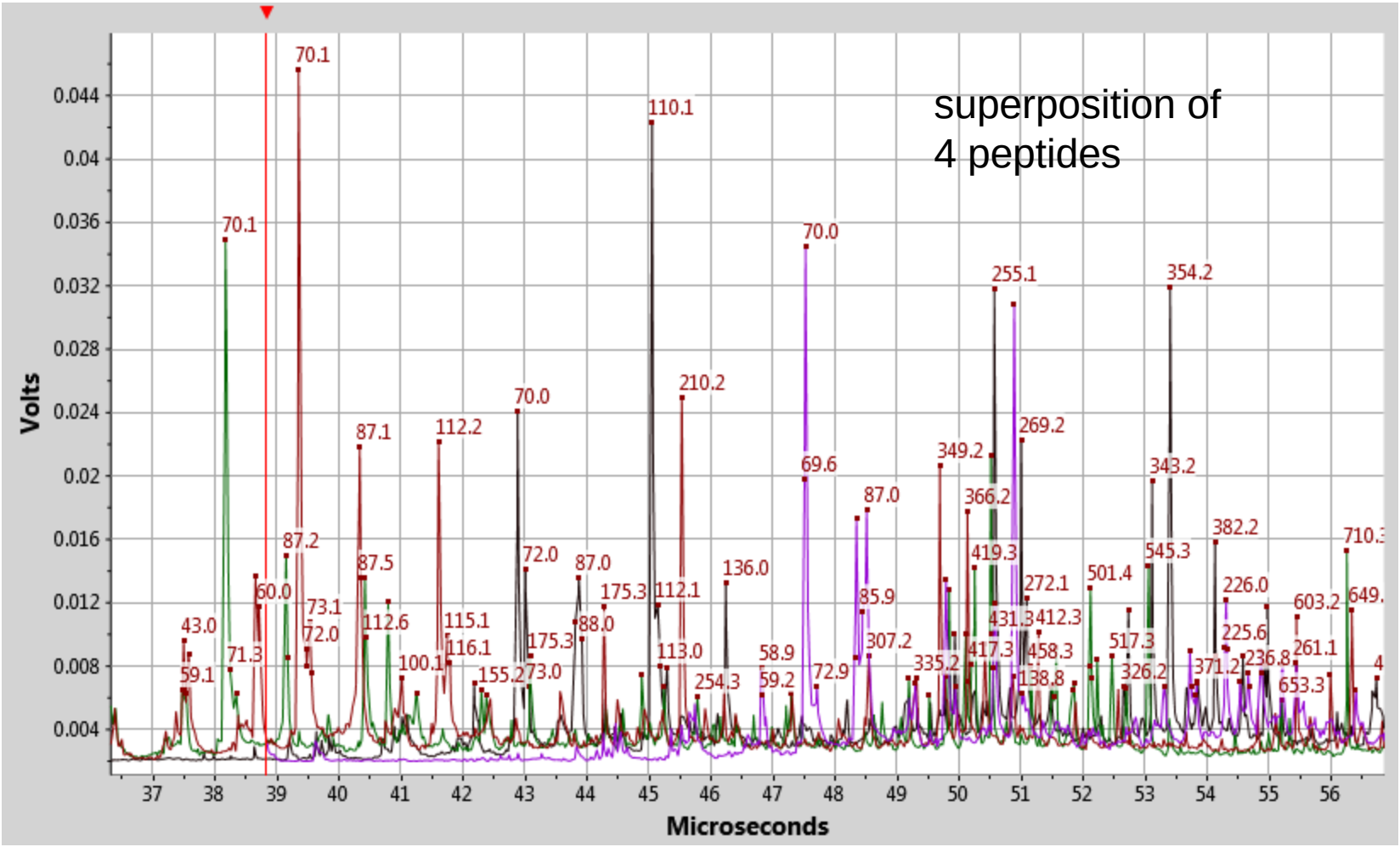
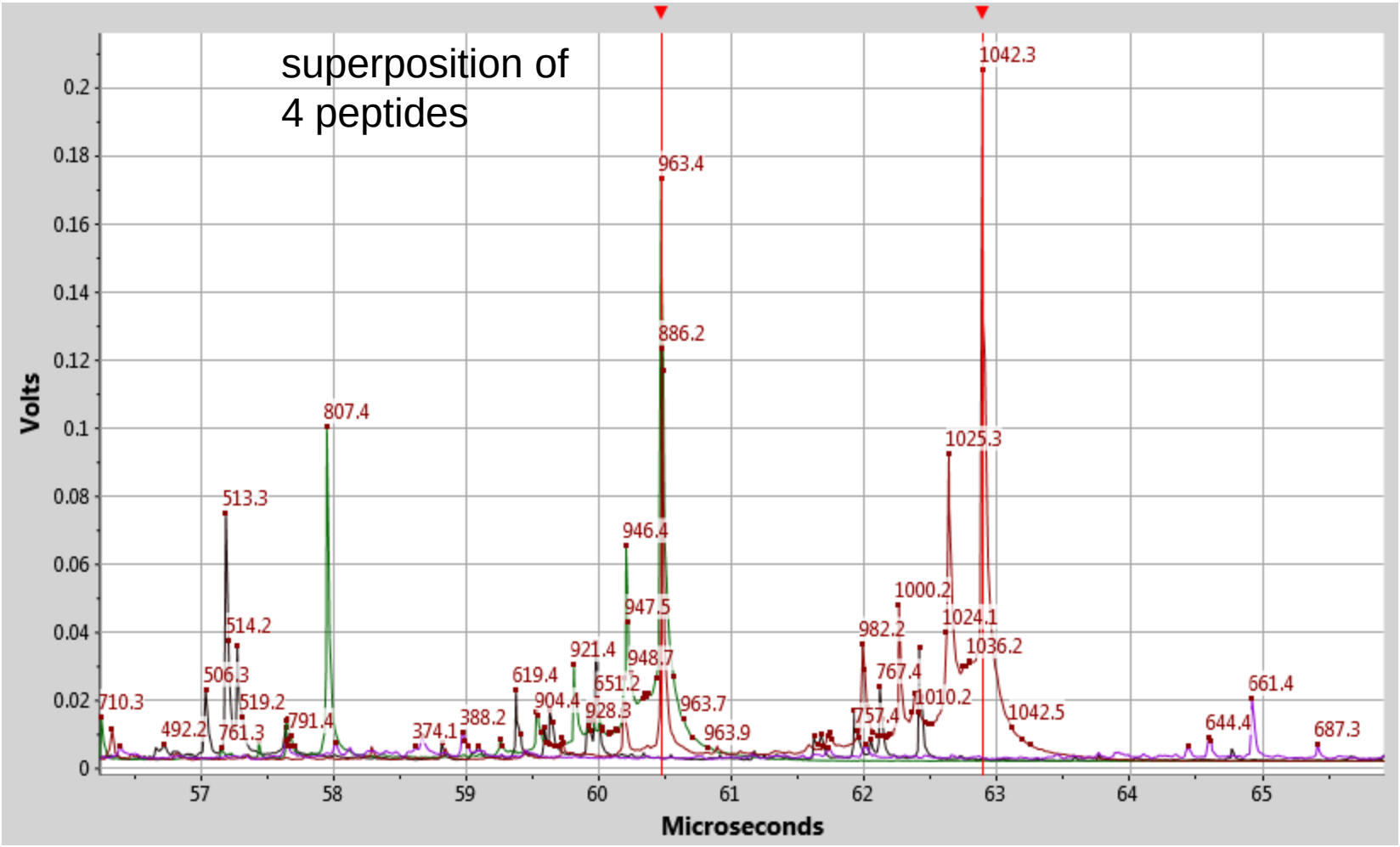

superposition of
4 peptides
Volts
Microseconds
0.044
0.04
0.036
0.032
0.028
0.024
0.02
0.016
0.012
0.008
0.004
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
70.1
110.1
70.1
70.0
255.1
354.2
210.2
70.0
87.1
112.2
269.2
349.2
69.6
343.2
87.0
366.2
382.2
87.2
87.5
72.0
87.0
136.0
419.3
545.3
501.4
272.1
226.0
60.0
73.1
175.3
112.1
85.9
603.2
43.0
112.6
115.1
88.0
431.3
412.3
72.0
175.3
307.2
225.6
71.3
116.1
113.0
58.9
417.3
458.3
517.3
261.1
100.1
59.1
155.2
73.0
254.3
59.2
72.9
335.2
138.8
326.2
371.2
236.8
653.3


superposition of
4 peptides
Volts
Microseconds
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
57
58
59
60
61
62
63
64
65
1042.3
963.4
886.2
807.4
1025.3
513.3
946.4
1000.2
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1024.1
514.2
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1036.2
921.4
948.7
506.3
619.4
767.4
651.2
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904.4
1010.2
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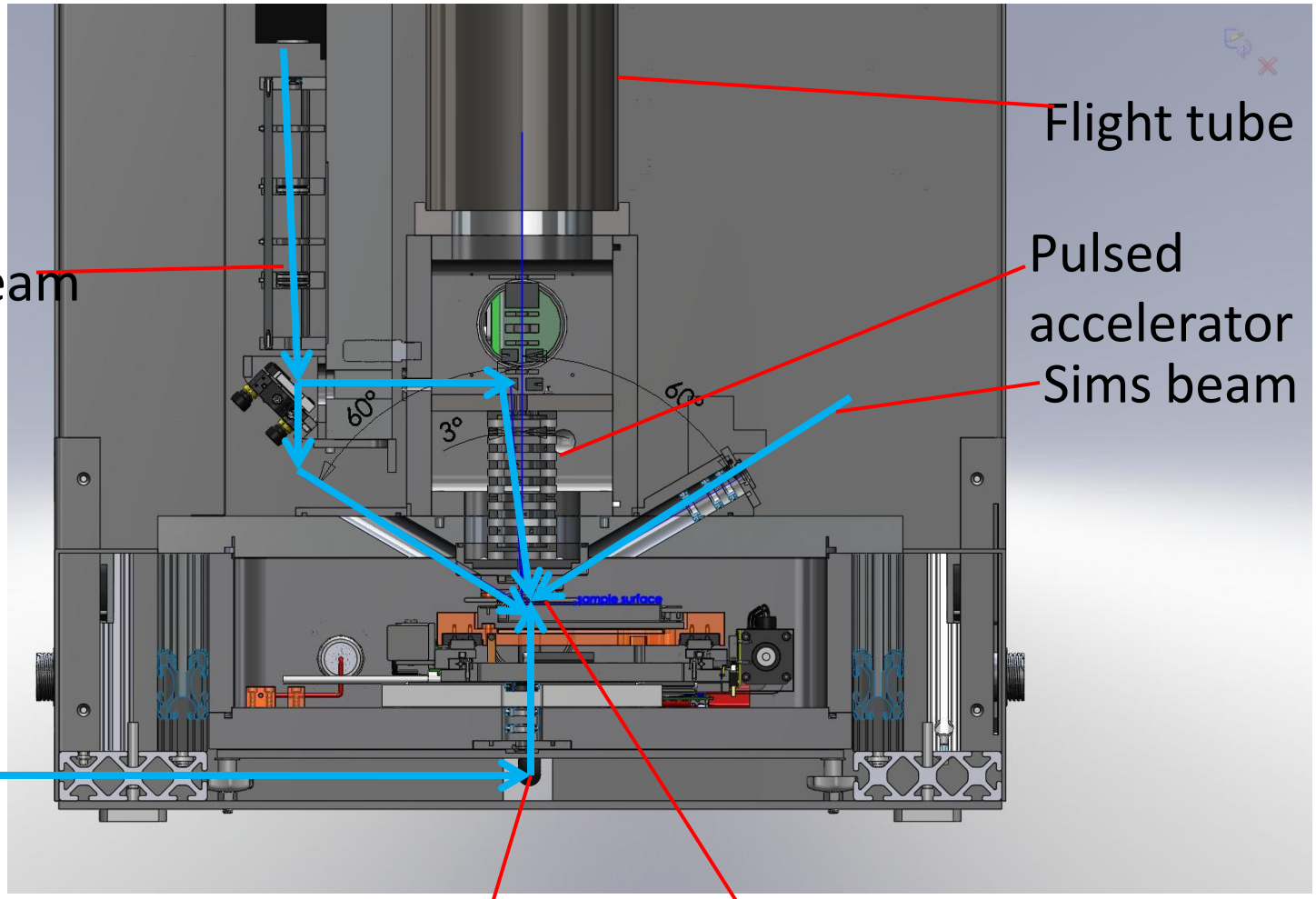
963.9


Flight tube
laser beam
Pulsed accelerator
Sims beam
60°
3°
60°
sample surface
Backside illumination
Sample plate

Spatial Resolution and Speed

- Angle min spot(μm) max scan rate at max res(s/mm^2)*

normal	25	16@ 5mm/s
30 deg	2.5	1600@ 0.5mm/s
180 deg	1	10000 @ 0.2 mm/s

*50 laser shots/spectrum @5kHz

50 kHz laser now available and may enable 10x further speed

Acknowledgements

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  - NIGMS and NCRR of NIH
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