Accurate determination of protein profiles in complex biological samples by MALDI-TOF MS

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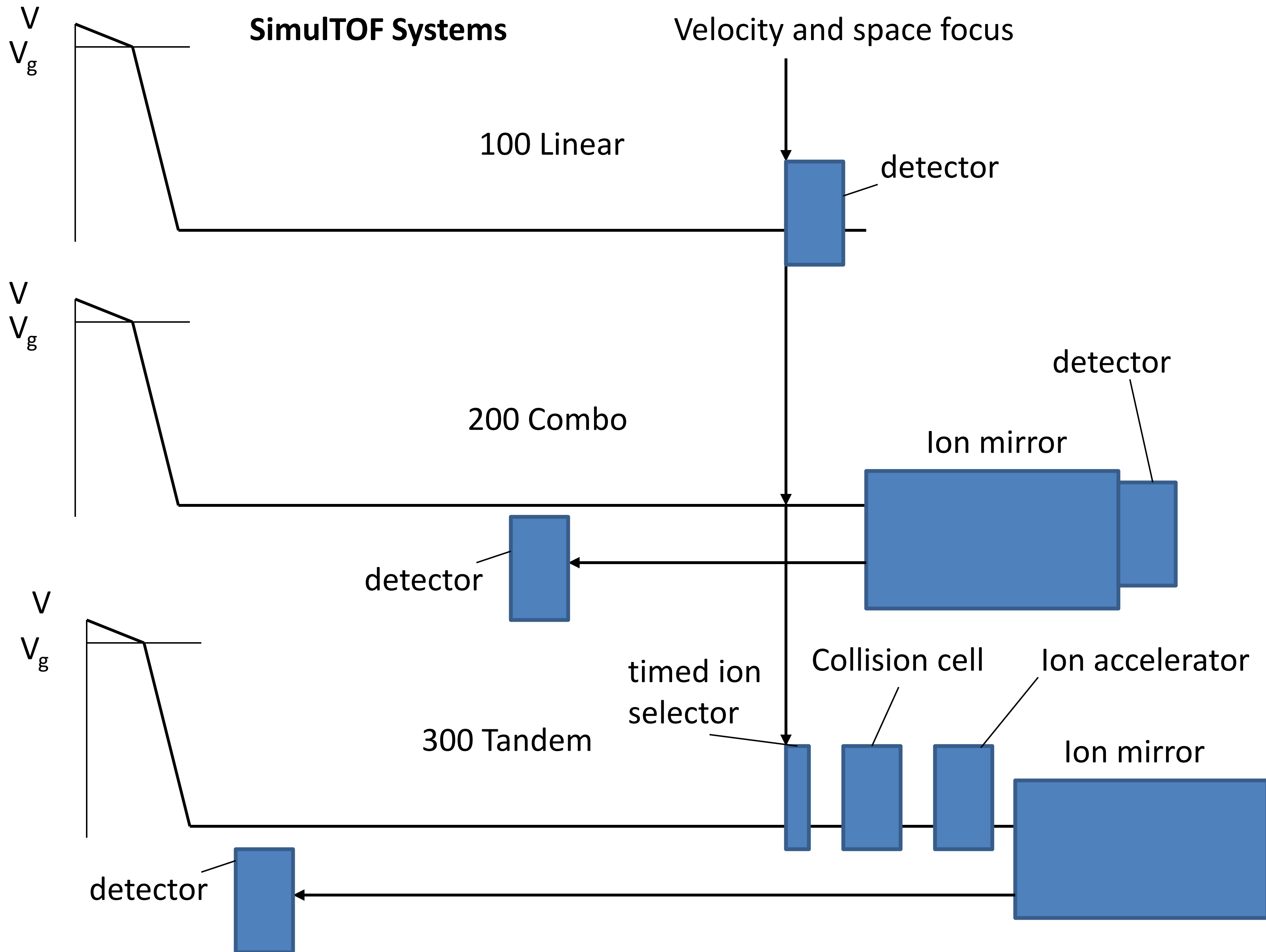
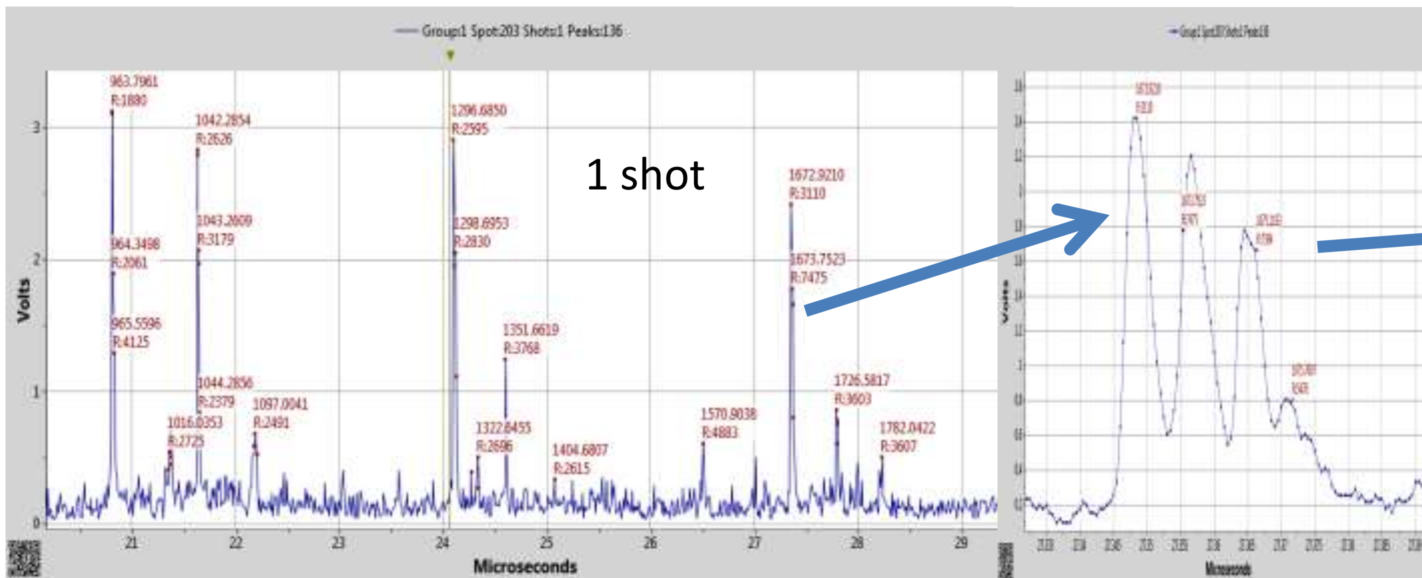
SimulTOF Systems

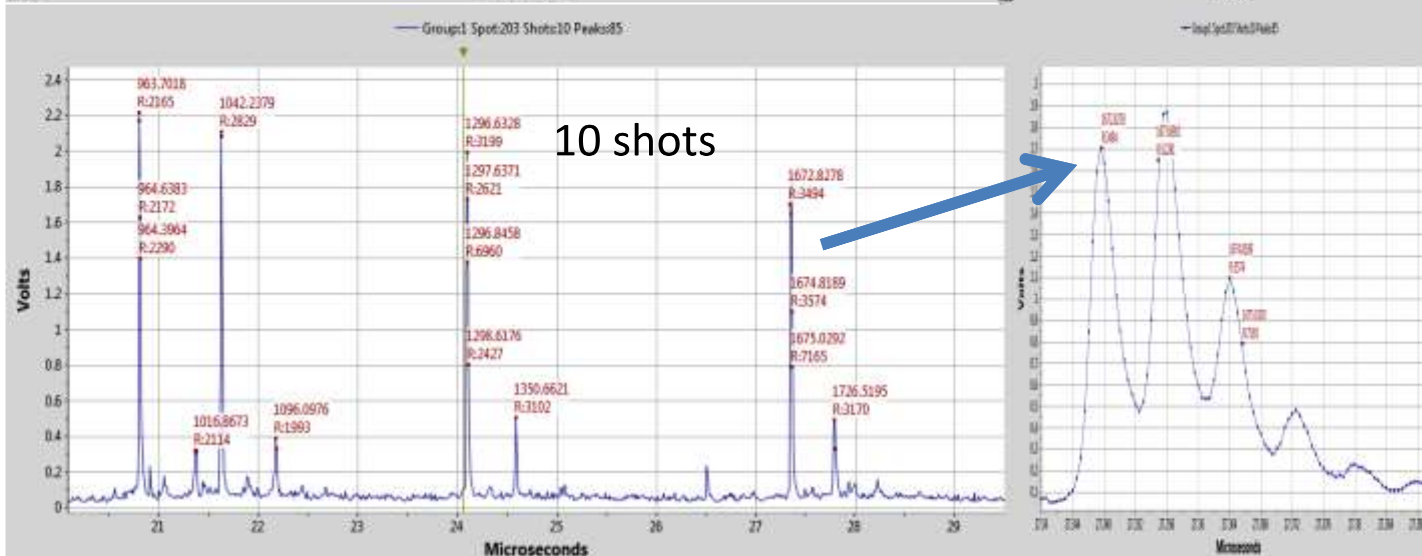
Lunchtime Workshop
MSACL
Tuesday, March 4, 2014

Commercial products introduced at ASMS 2012


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



~24,000 ions detected

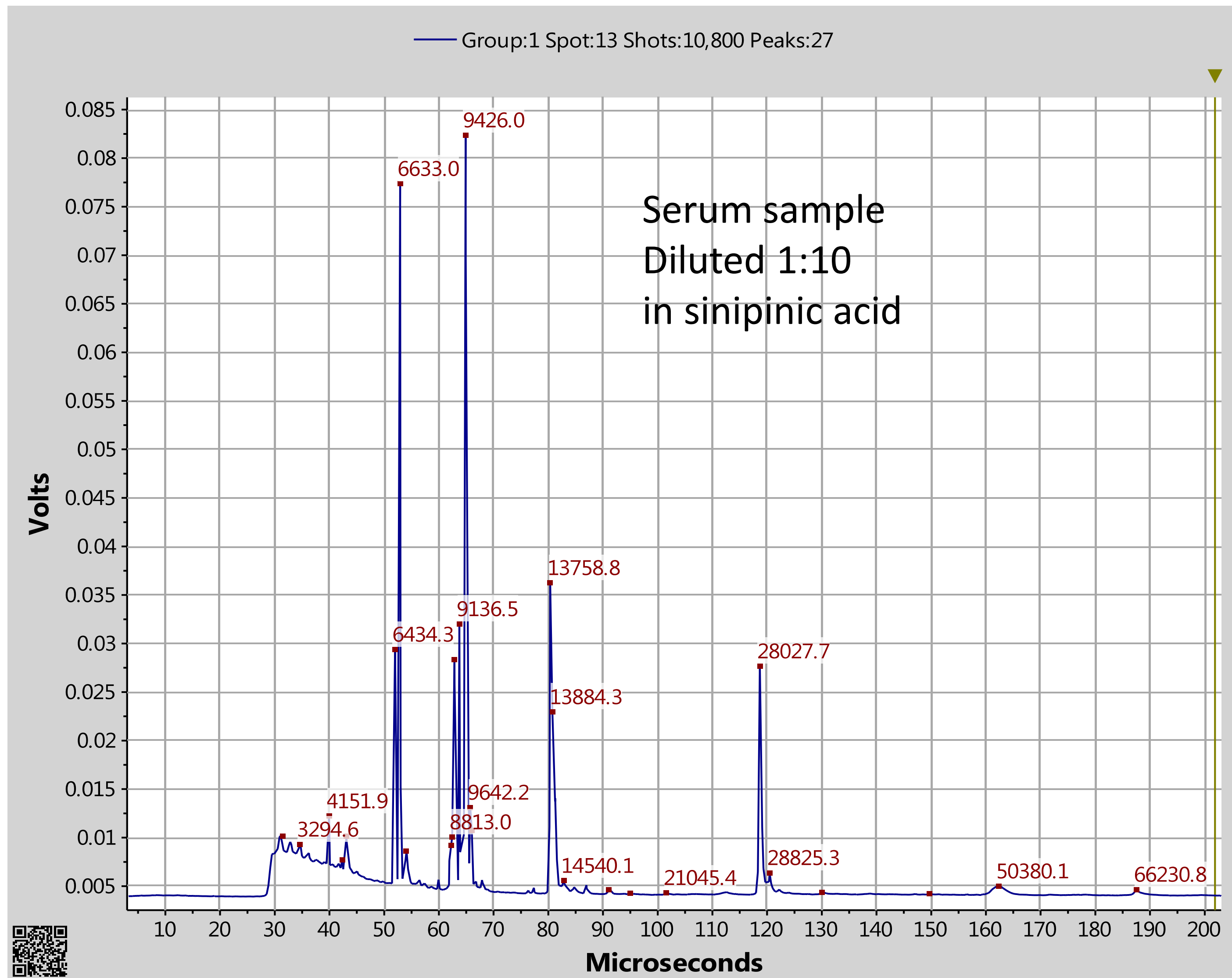


10% over all efficiency

Mixture of peptide standards, 100 femtomole/µL, 2.5 mm spot

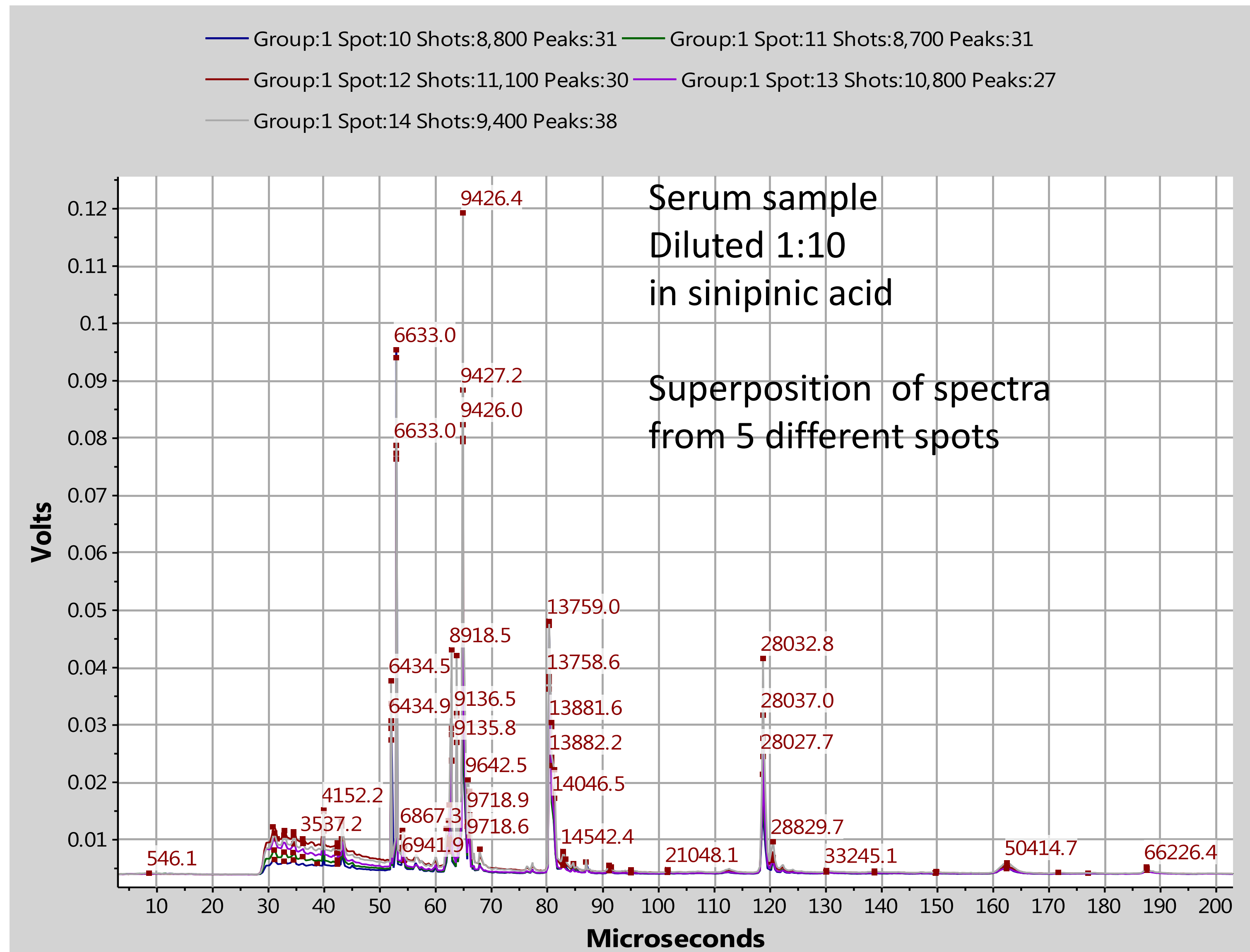
Laser spot ca. 50 µm, fluence 1.3x threshold, 0.4 attomole/spot=240,000 molecules

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

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

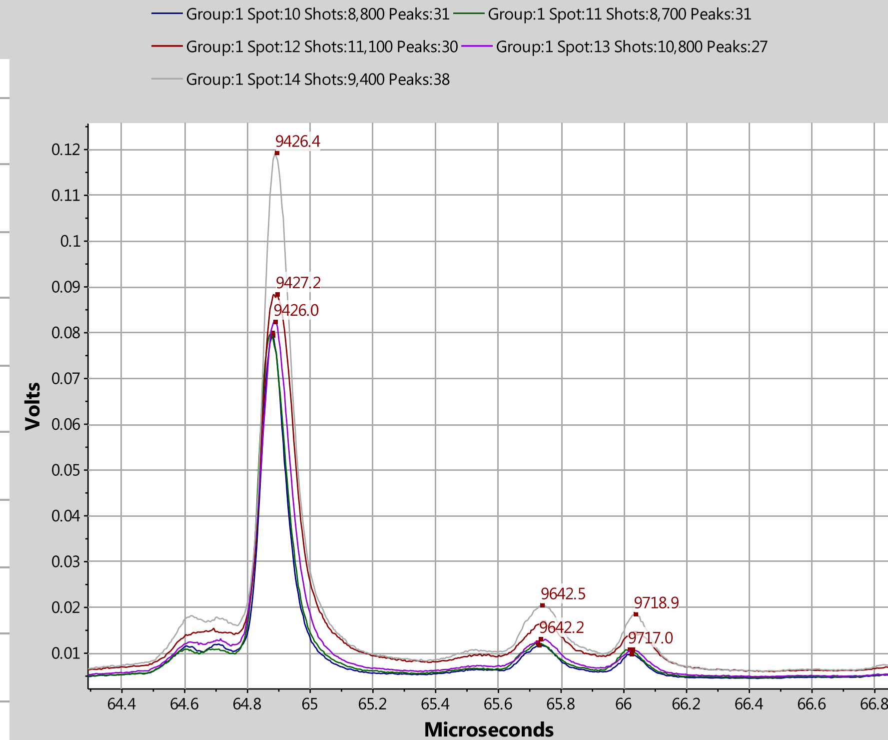
Volts

0.12 0.11 0.1 0.09 0.08 0.07 0.06 0.05 0.04 0.03 0.02 0.01

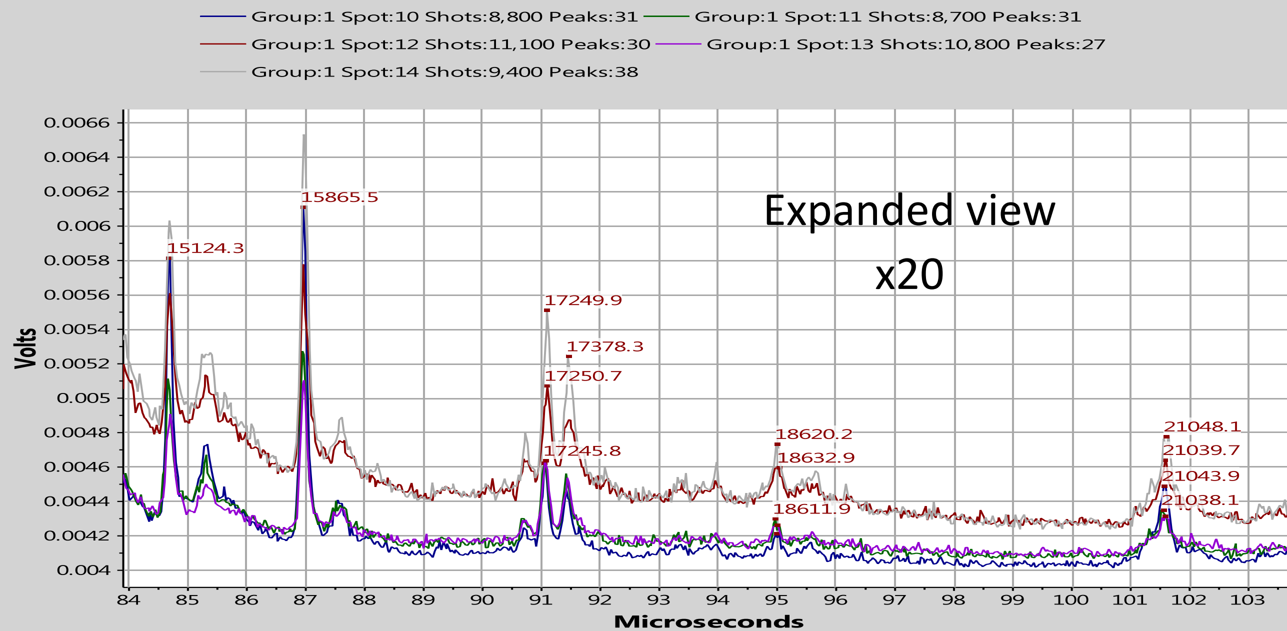
9426.4
9427.2
9426.0
8918.5
9136.5
8920.4
9136.5
9135.8
8917.2
9642.5
8814.1
8687.7
8813.0
9718.9
9718.6
10205.6

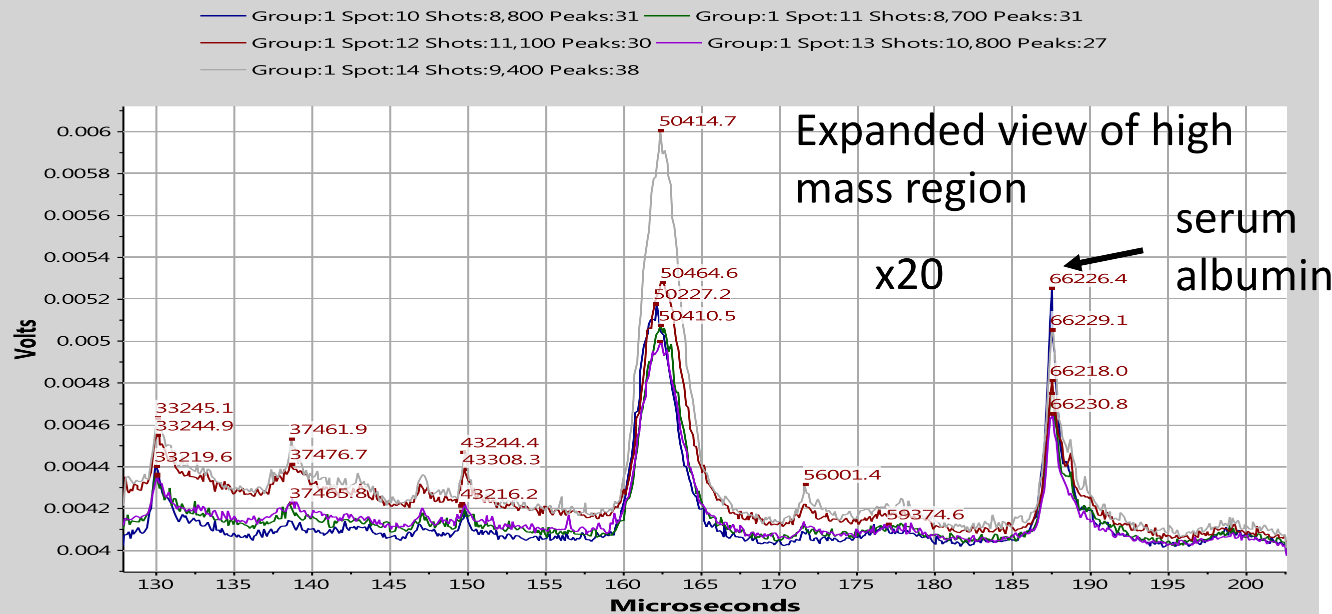
61 62 63 64 65 66 67 68 69

Microseconds



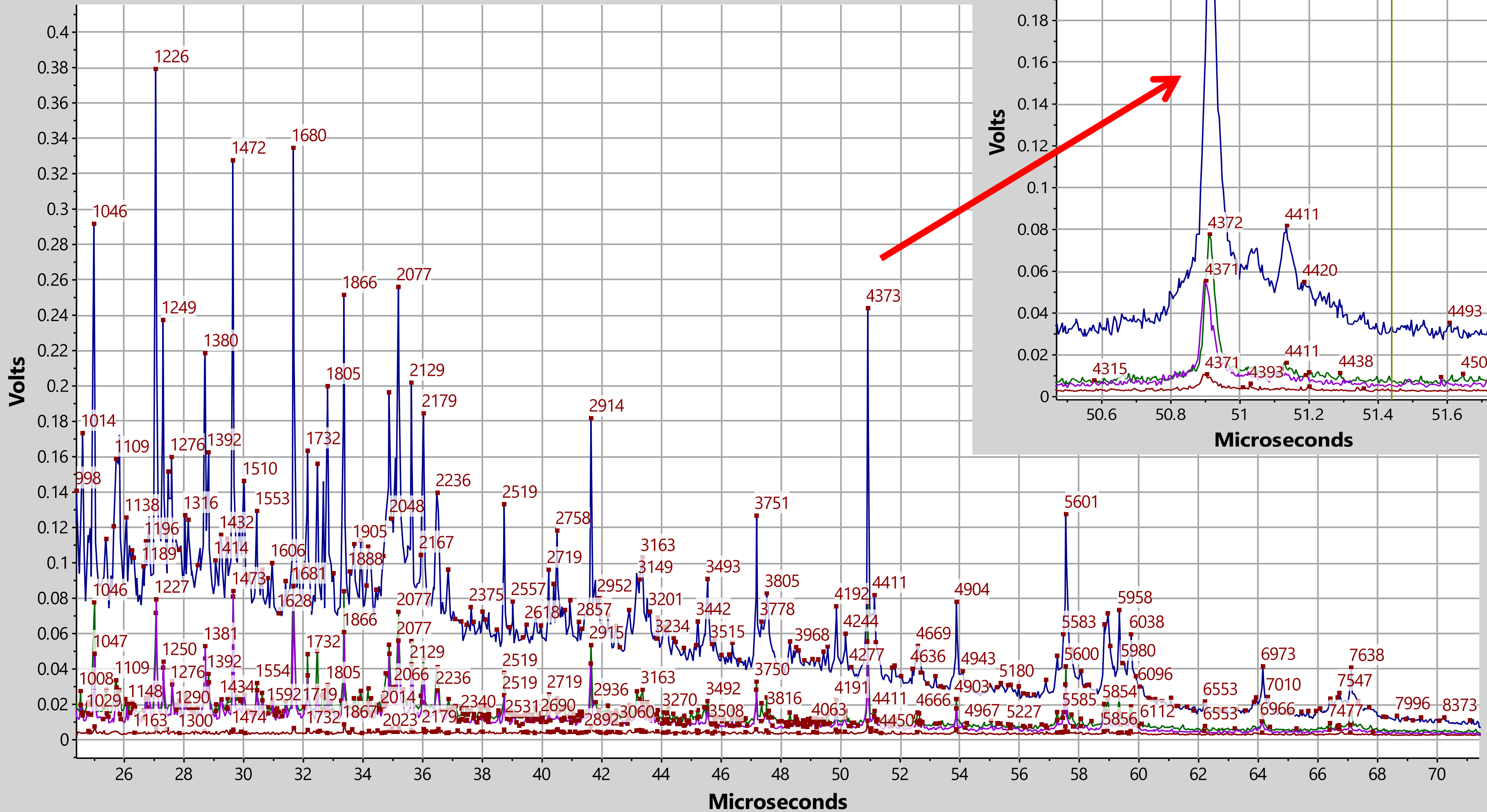
These are raw data. No normalization, background subtraction, smoothing or other data processing has been employed.


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
Volts
Microseconds
15124.3
15865.5
17249.9
17378.3
17250.7
17245.8
18620.2
18632.9
18611.9
21048.1
21039.7
21043.9
21038.1


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
Volts
Microseconds
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

Conventional Wisdom

- MALDI
- is Not
- Quantitive



4 spectra 50 shots ea. On one sample spot
Intensity varies by factor of 20
@10 Hz requires 20 s

Your Old MALDI

How do we make MALDI Quantitative????

Data Acquisition, One Example

Data is acquired over mass range 1000-20,000 Da using 2 ns bins
2 kHz laser rate
50 shots averaged per spectrum
1 mm/s snake raster for 5 passes at 500 mm over 3 mm dia spot
Total travel 12 mm in 12 s generating 480 spectra with 50 μm resolution
Spectra with no significant peaks are not saved
Only 10% of sample used, 240,000 total laser shots possible

Data Processing (SimulTOF Wizard)

Spot Average
Baseline Correction
Smooth
Calibrate and Detect Peaks
Normalize
Bin by mass rather than time
Quantify
Report

Peak table includes:
Intensity (number of ions/peak)
Mass
standard deviation
(square root of number of ions/peak)
Resolving power =m/dm=mass/FWHM

Mass Binning

Data is binned into mass bins using the following form:

$m_{n+1}=m_n(1+r)$, $m_n=m_0(1+r)^n$, $m_N=m_0(1+r)^N$,
m_0 is the first mass, m_N is the last mass, and N is the total number of bins.
$Log(m_N/m_0)=N[log(1+r)]$, $r=10^{[x/N]}-1$, where $x=log(m_N/m_0)$,
for example, if $m_N/m_0)=20$, $R=1/r=m/Dm=400$
Then N=1200

One approach is to detect peaks in the unknown spectrum
Assign peaks to bins and sum intensity of all peaks in the bin.

An alternative approach is to sum intensity in each mass bin
and use this intensity as that for each bin.

The challenge is to determine which approach is best at minimizing
instrumental artifacts due to such problems as incomplete separation
of adjacent peaks.

We have evaluated performance with R=400 and R=2000

Comparing Spectra

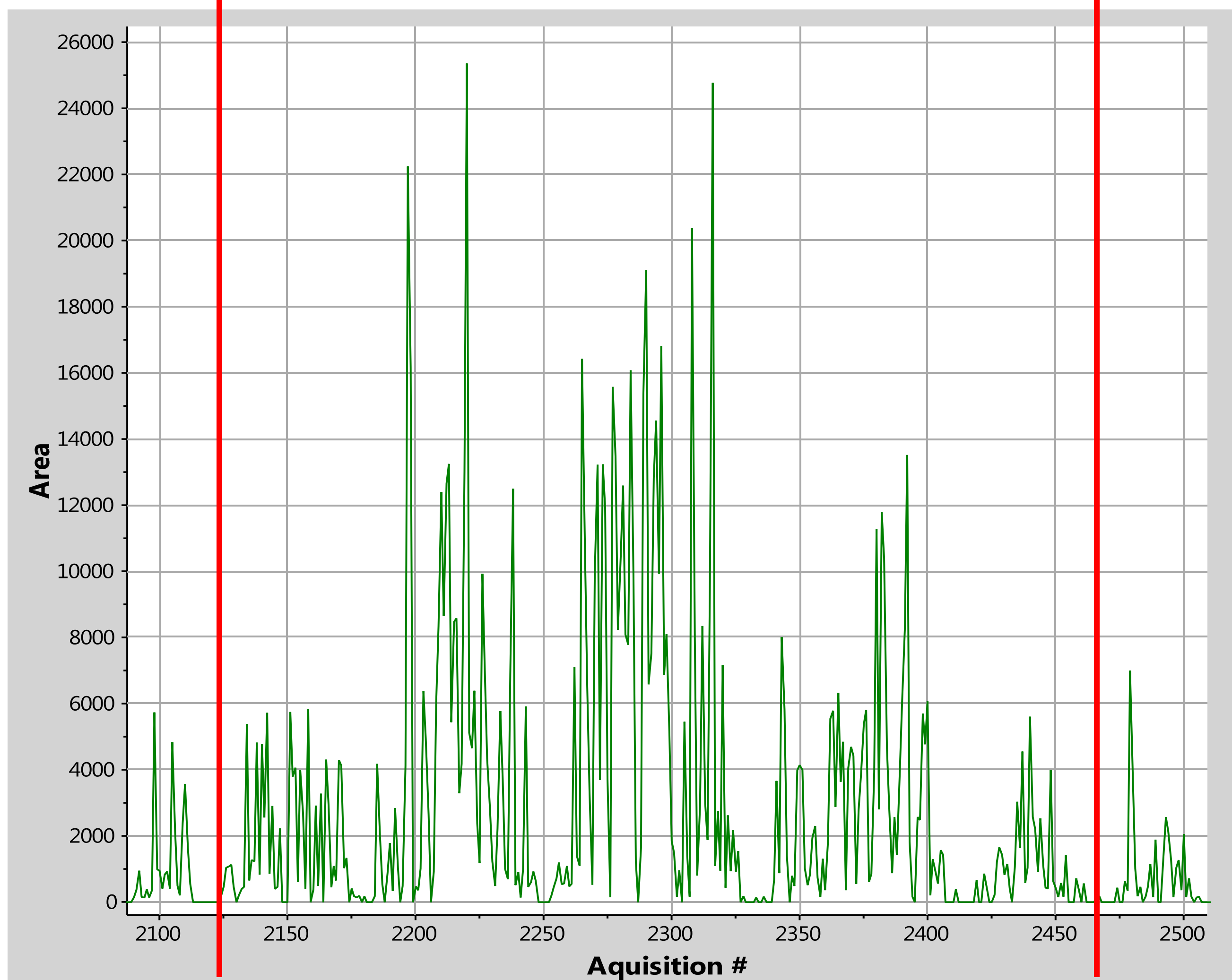
Use standard vector algebra on normalized and binned spectra

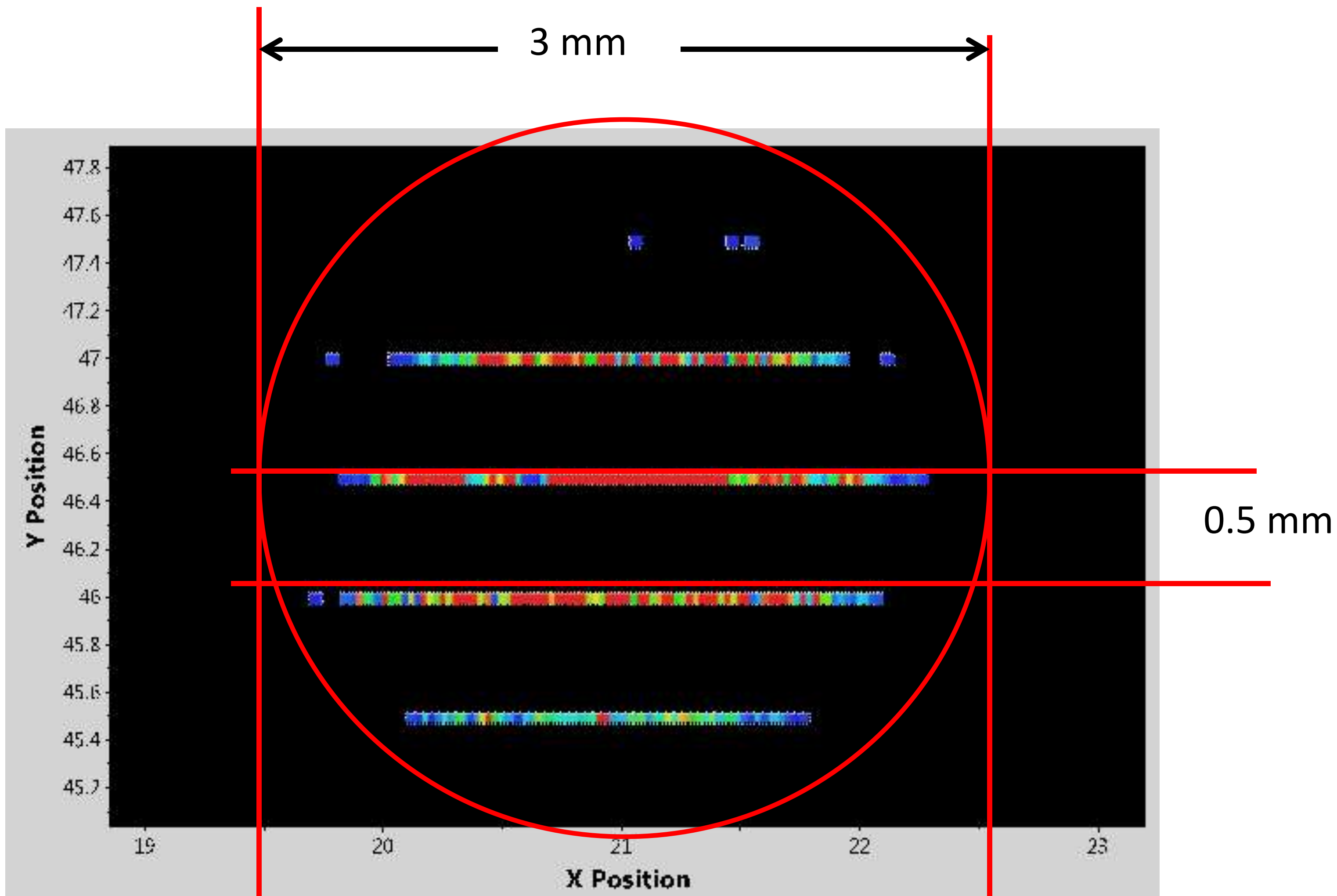
Difference in spectra computed by

$$(N_a - N_b)/\text{Square Root}(N_a + N_b)$$

For each bin

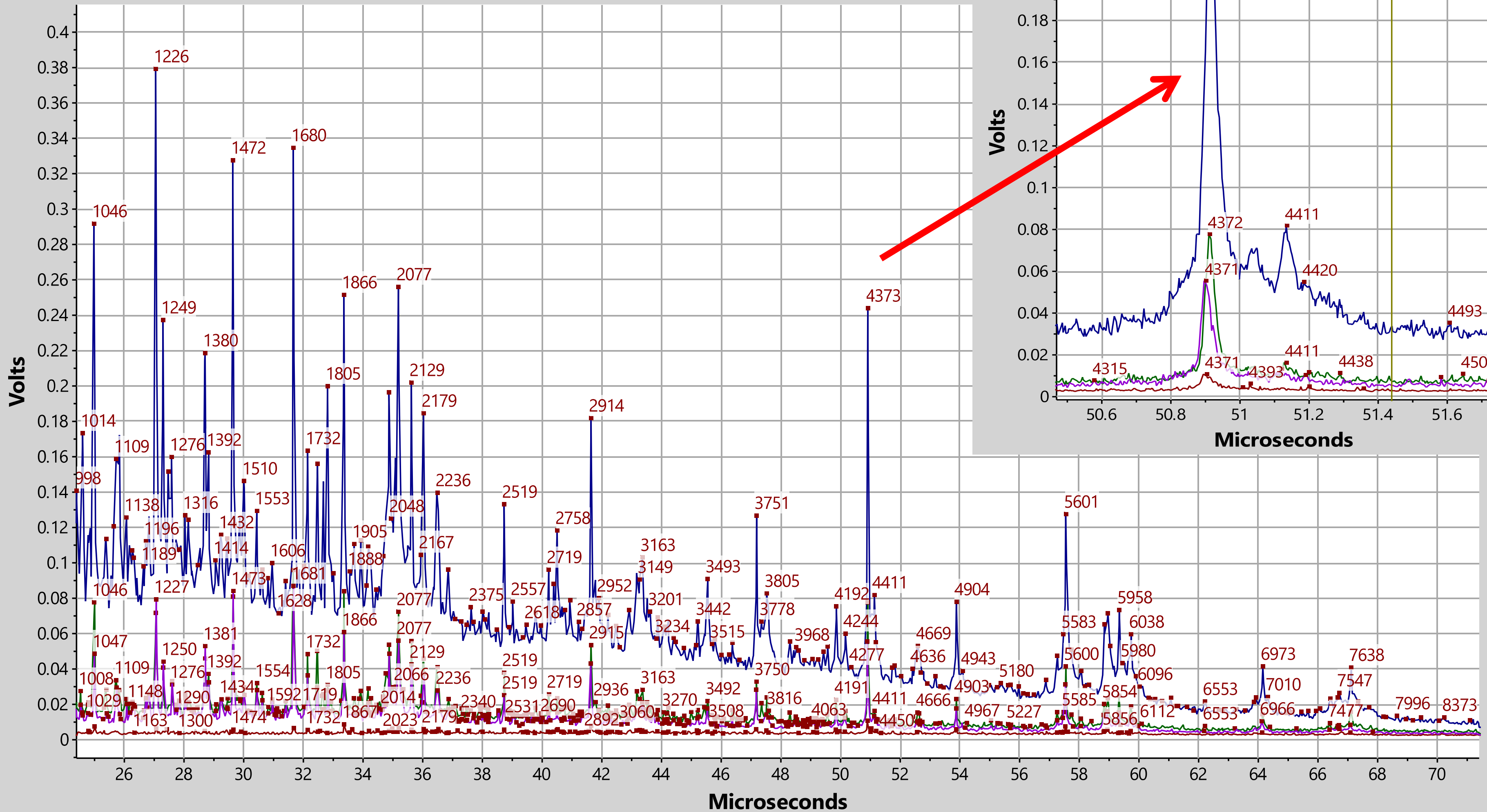
347 spectra 50 shots ea.
12s @2000 Hz





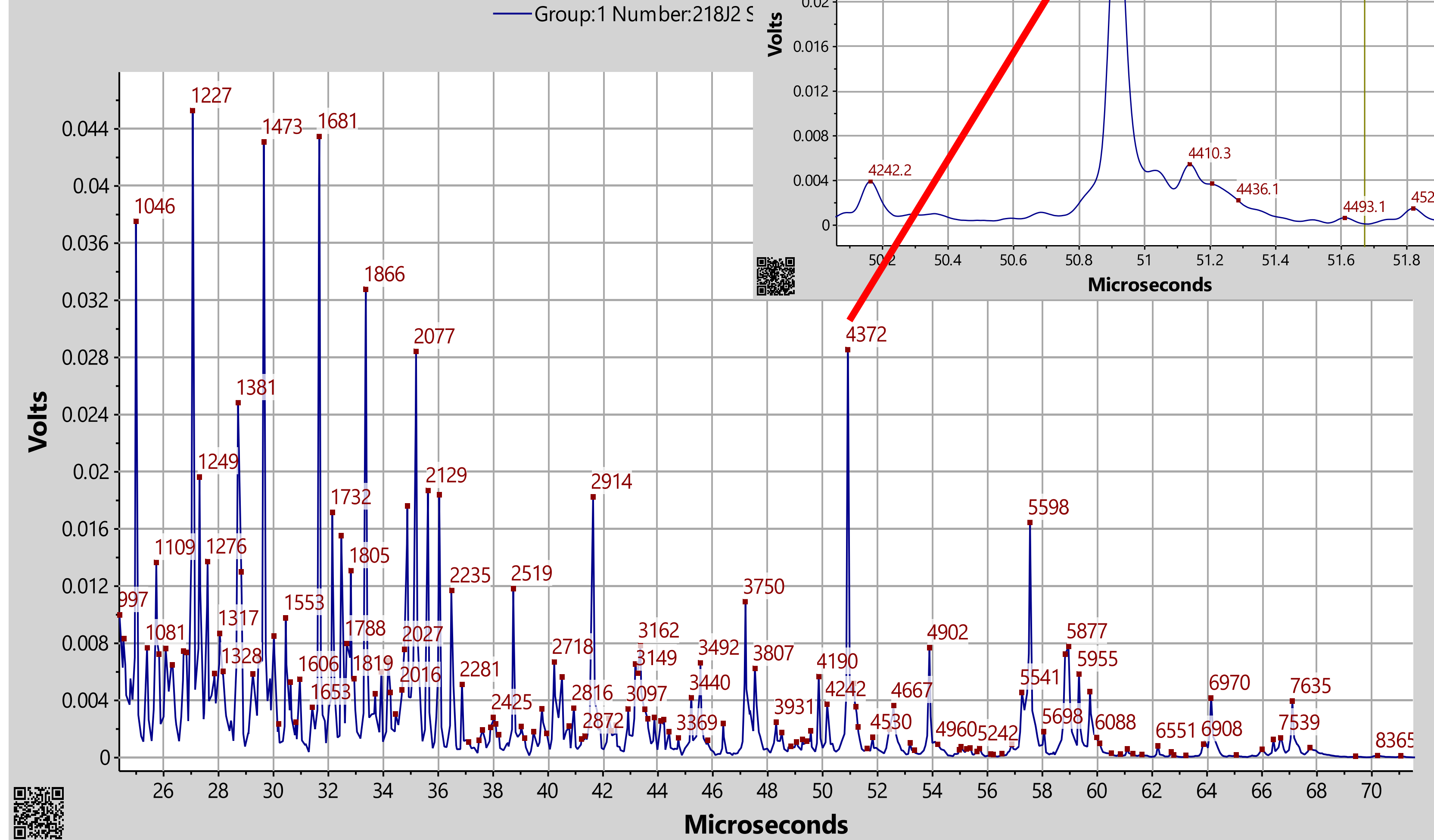
Spot 176

17,400 shots recorded out of 24,000 shots applied
10% of sample scanned in 12 s



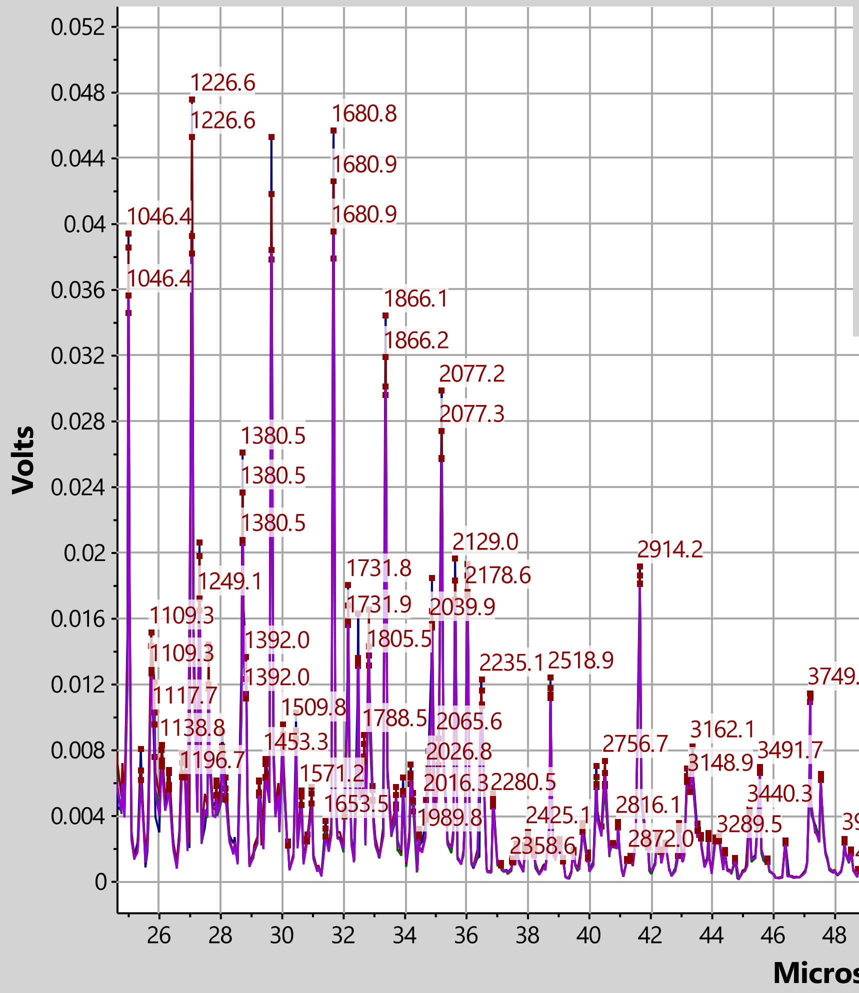
4 spectra 50 shots ea. On one sample spot
Intensity varies by factor of 20
@10 Hz requires 20 s

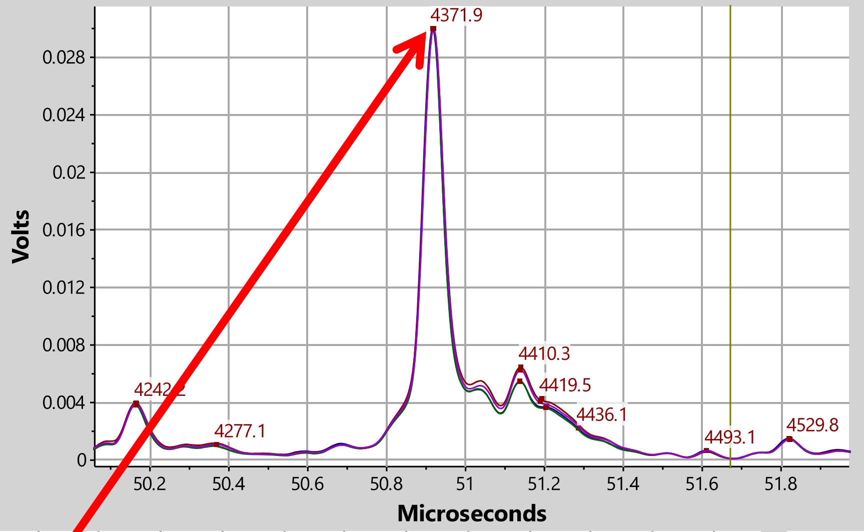
Your Old MALDI



Processed spectrum from same sample spot as above

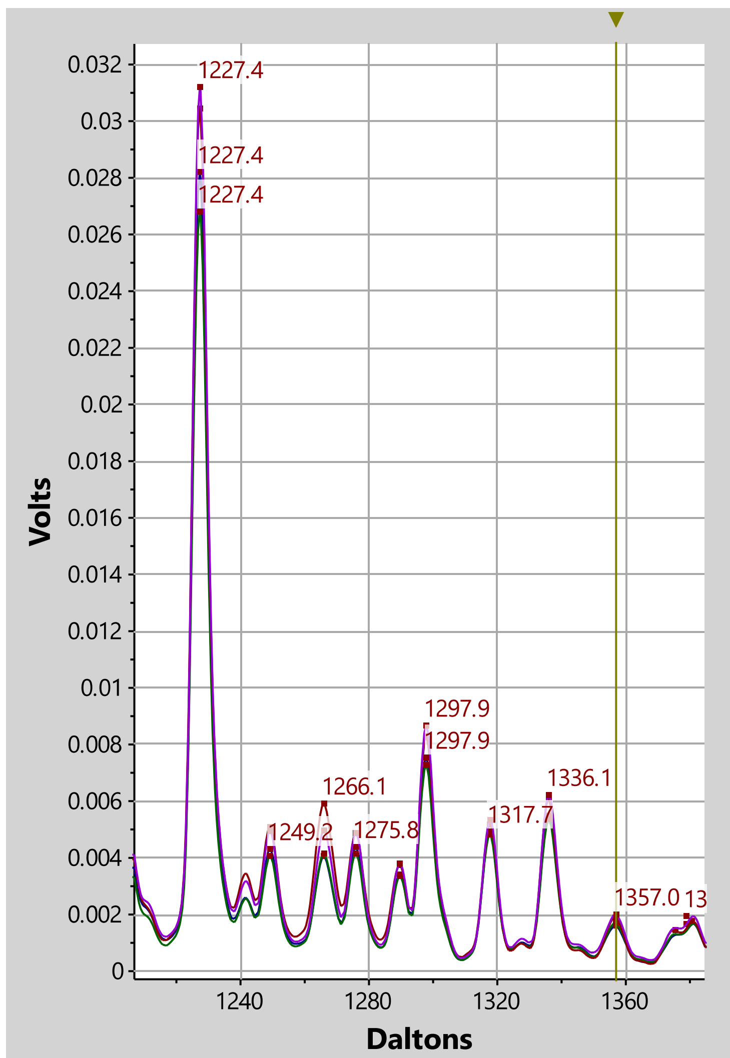
Our New MALDI



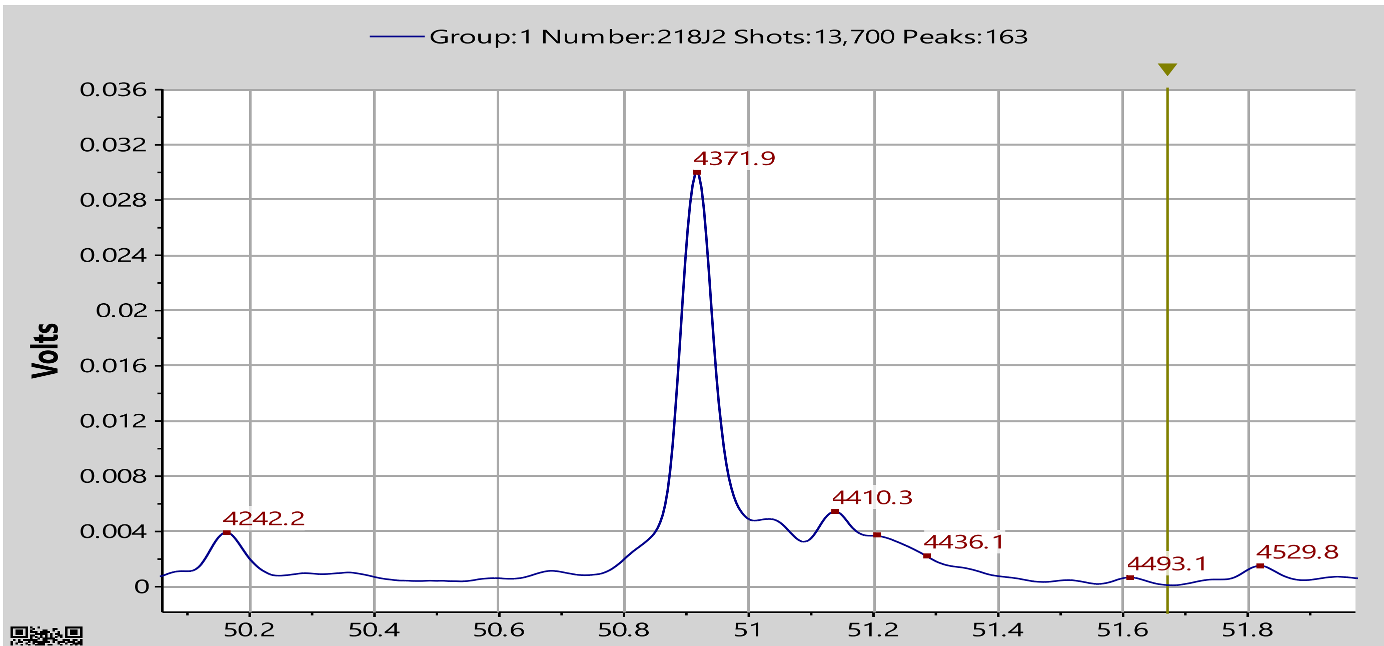


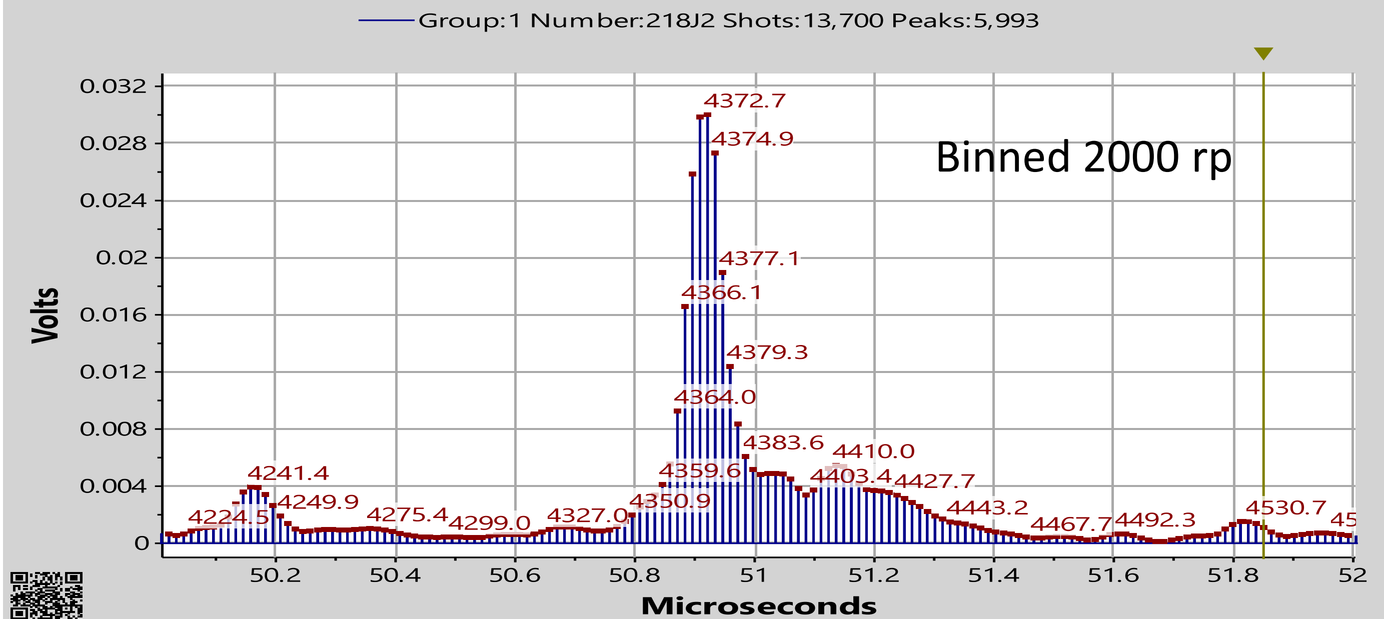
Spectra from 4 different sample spots super imposed

Our New MALDI



Volts
Daltons
9099.2
9099.2
9099.3
9006.4
9480.7


Group:1 Number:218J2 Shots:13,700 Peaks:163
Volts
0.036
0.032
0.028
0.024
0.02
0.016
0.012
0.008
0.004
0
4371.9
4242.2
4410.3
4436.1
4493.1
4529.8
50.2
50.4
50.6
50.8
51
51.2
51.4
51.6
51.8


Group:1 Number:218J2 Shots:13,700 Peaks:5,993
Binned 2000 rp
Volts
0.032
0.028
0.024
0.02
0.016
0.012
0.008
0.004
0
4372.7
4374.9
4377.1
4366.1
4379.3
4364.0
4383.6
4410.0
4359.6
4403.4
4427.7
4241.4
4249.9
4224.5
4275.4
4299.0
4327.0
4350.9
4443.2
4467.7
4492.3
4530.7
50.2
50.4
50.6
50.8
51
51.2
51.4
51.6
51.8
52
Microseconds

Saliva Sample Prep

Individuals spit into tubes, ~ 11 AM. Participants DK, DP, KP, MV.

Spin 5 min.

Dilute 10 ul of supernatant into 90 ul of 5 mg/ml HCCA matrix in 75% acetonitrile / 0.1% TFA
Spot 2ul per well.

First set of Sample tubes saved in refrigerator at 4 C for 6 days. Not stirred

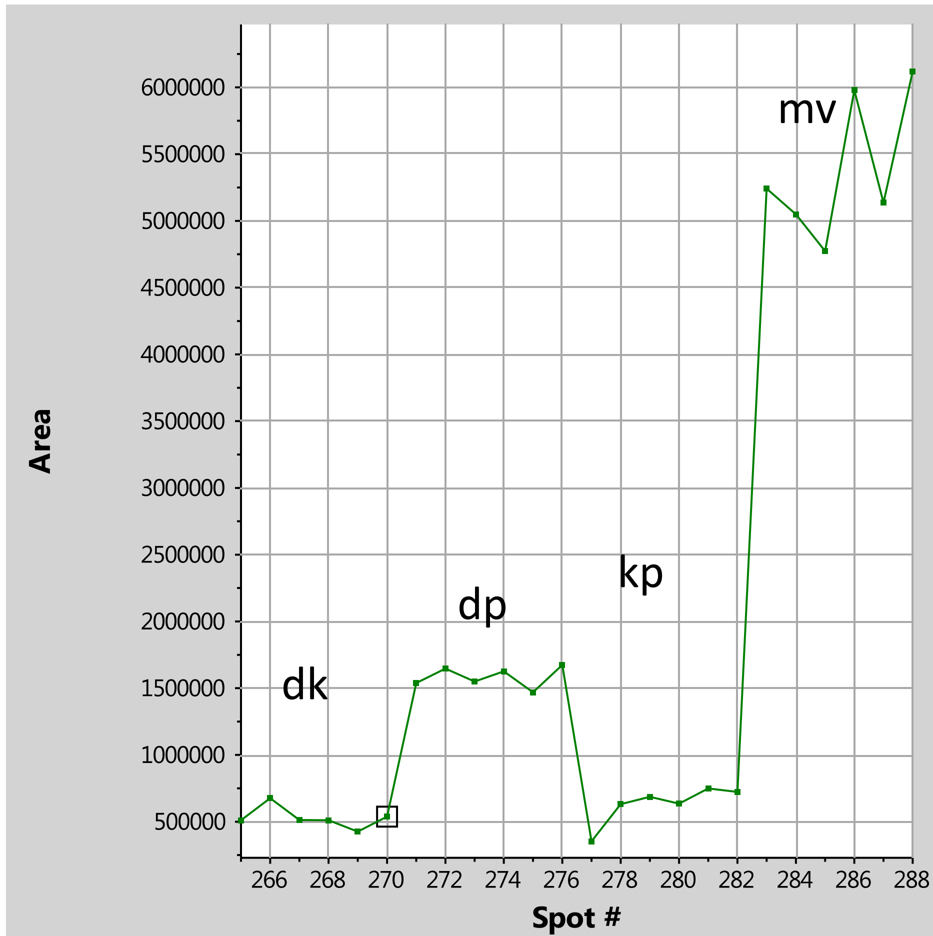
Fresh samples collected ~ 11AM.

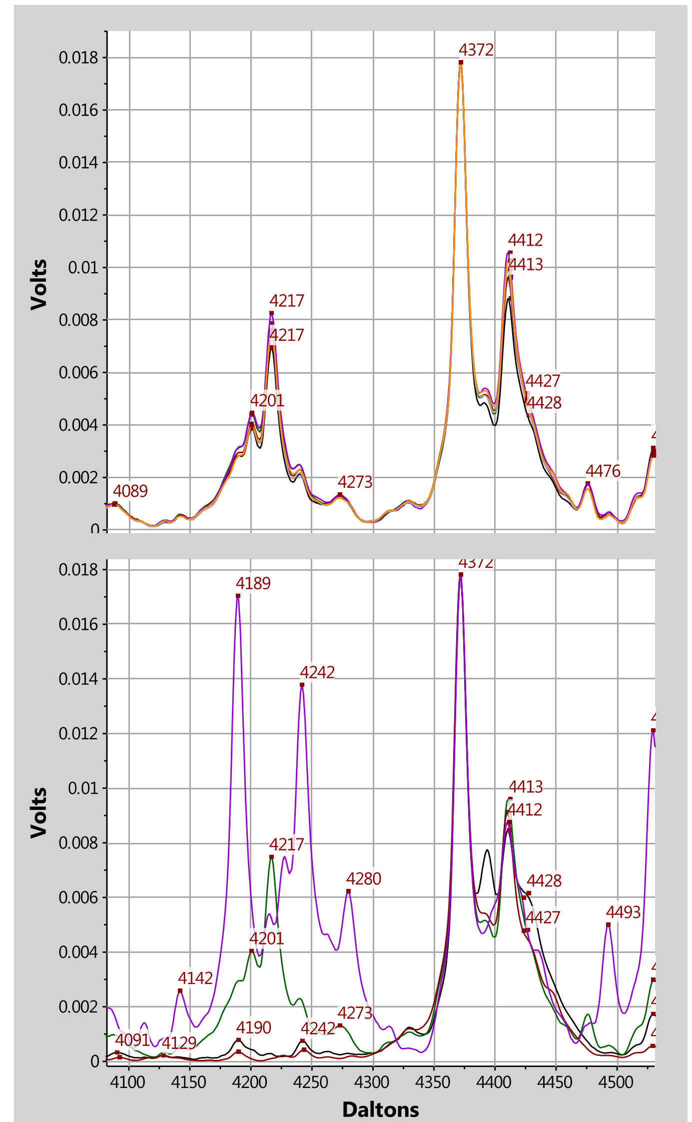
Spin again, repeat as above.

2 ul sample to 20 ul of matrix for rerun

Samples for DK, DP, KP, MV. 6 each.

dp





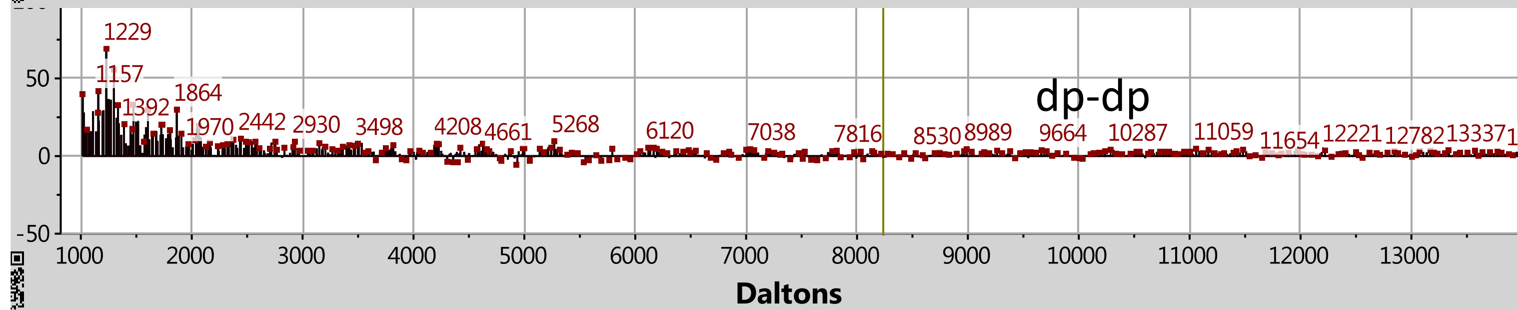
Normalized to TIC, 400 RP

Group:1 Spot:286 Shots:24,850 Peaks:1,200 File:L22

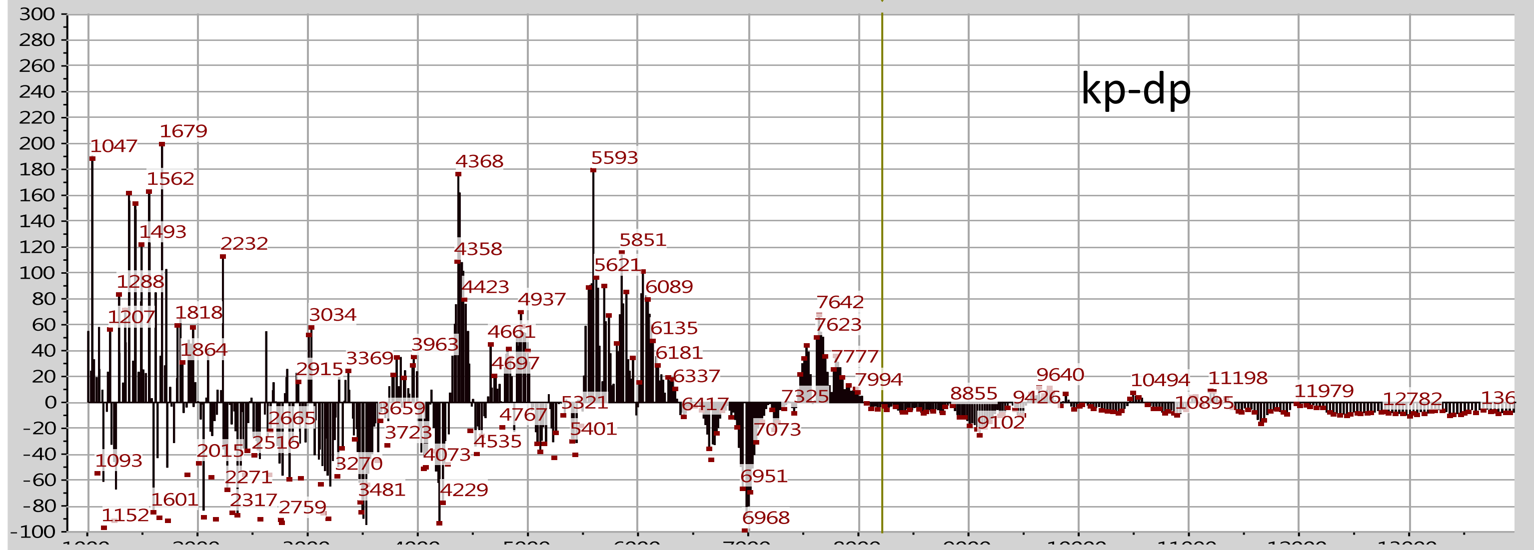
mv-dp

$\Delta N/\sigma$

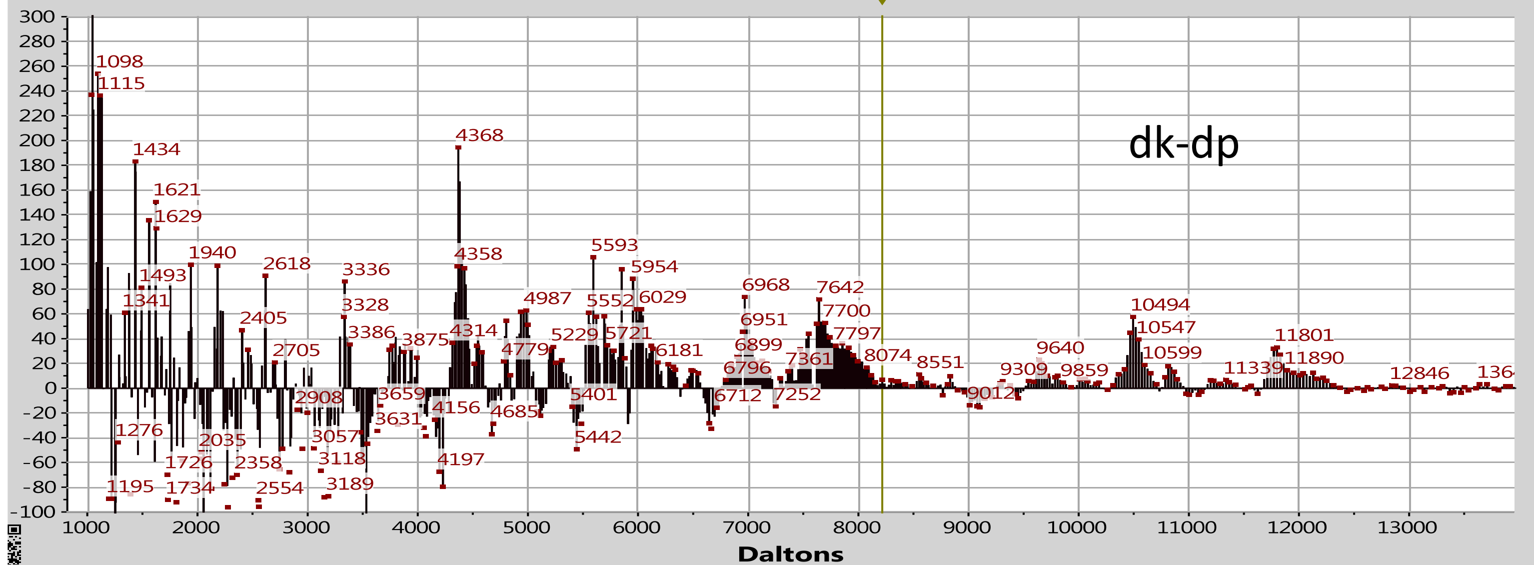
Daltons



Normalized to TIC, 400 RP

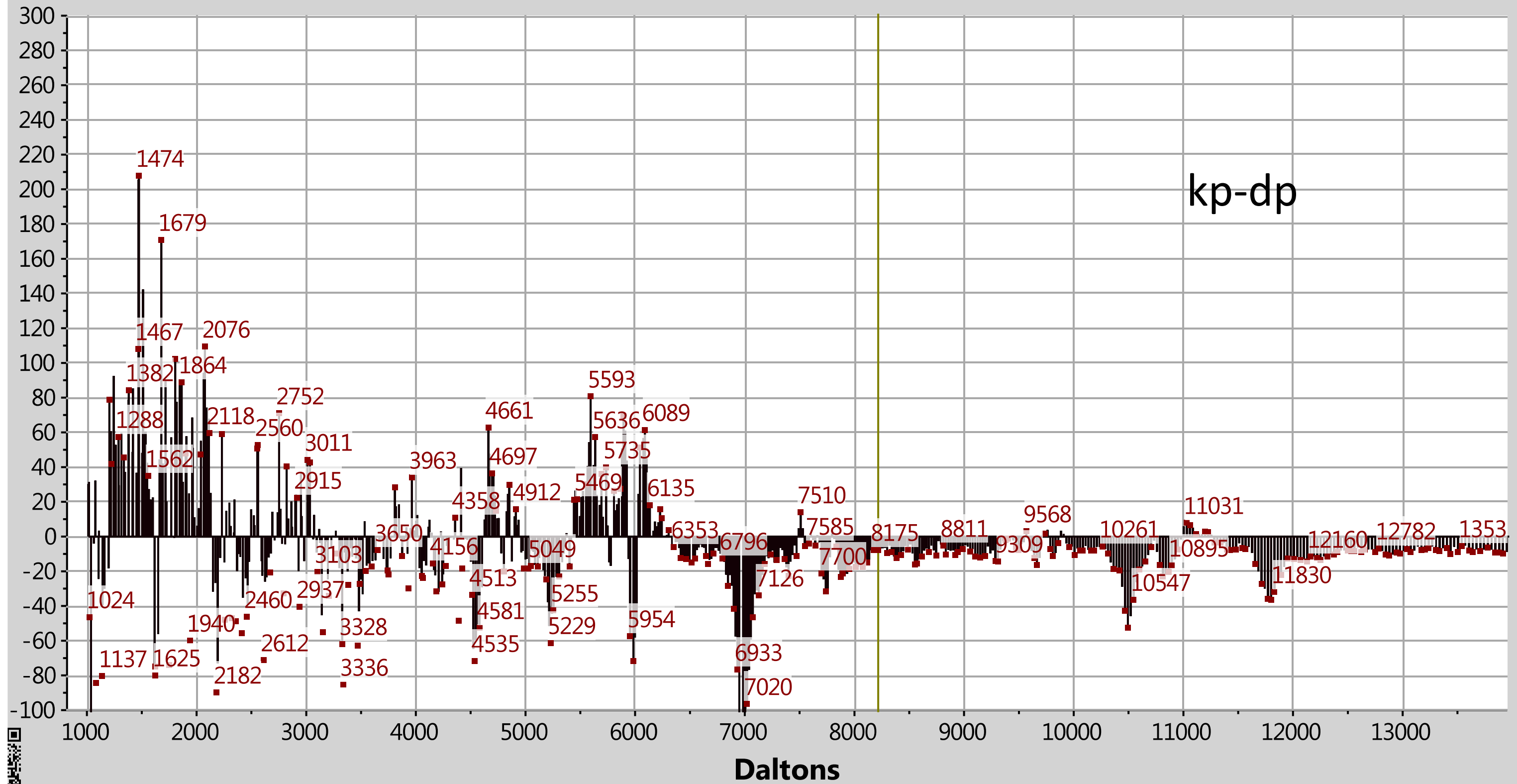


ΔN/σ



Normalized to TIC, 400 RP

ΔN/σ



Normalized to Base Peak, 400 RP

Group:1 Spot:274 Shots:18,450 Peaks:1,200 File:L10

dp-dp

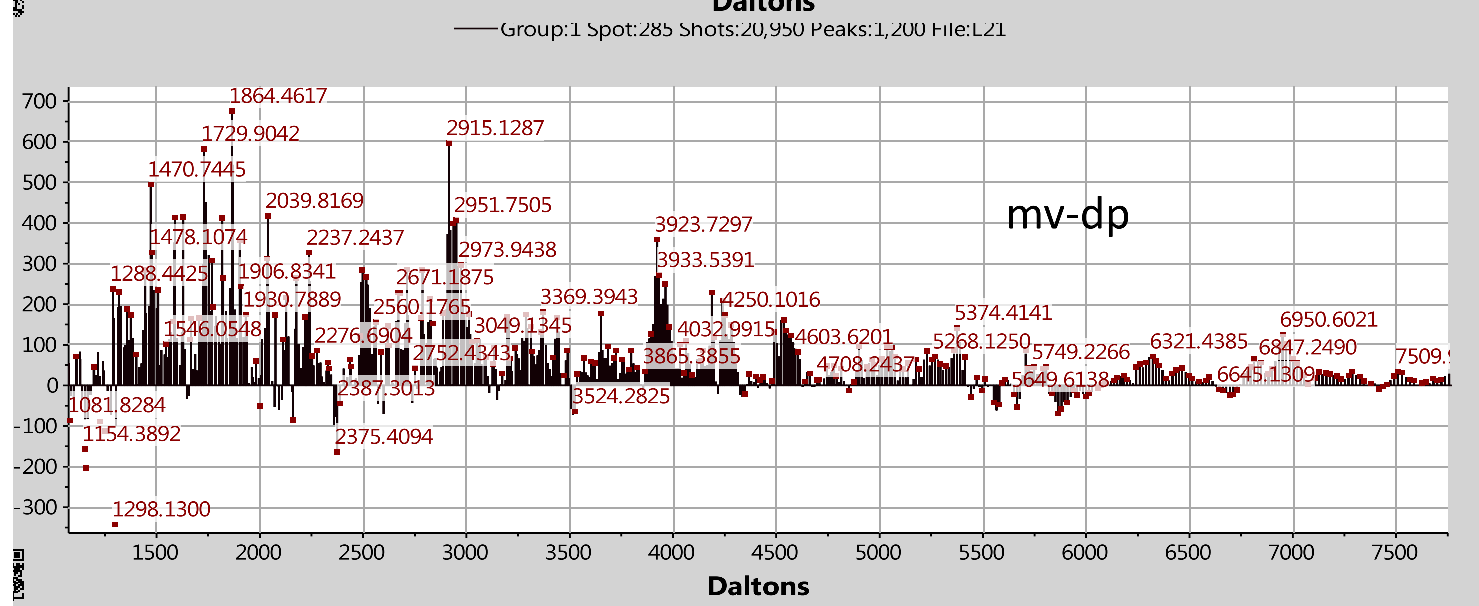
1269 1508 1805 2065 2375 2618 2930 3254 3524 3789 4208 4457 4744 4999 5268 5497 5792 6196 6481 6847 7216 7473

700 600 500 400 300 200 100 0 -100 -200 -300

1500 2000 2500 3000 3500 4000 4500 5000 5500 6000 6500 7000 7500

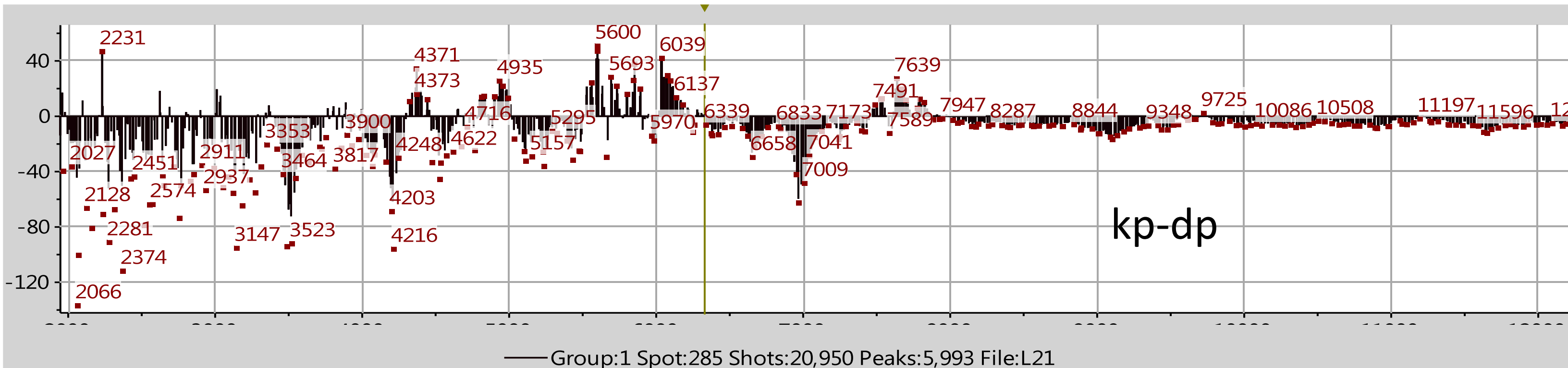
Daltons

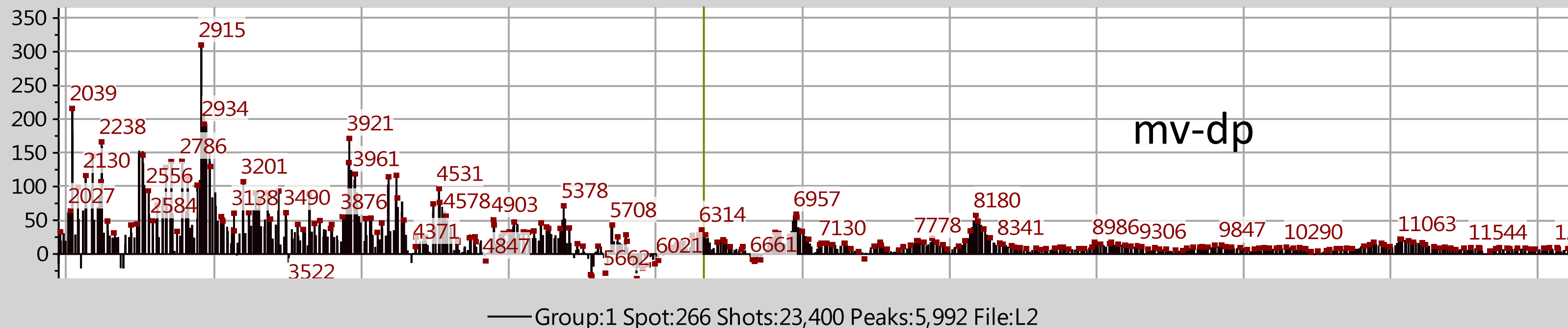
ΔN/σ

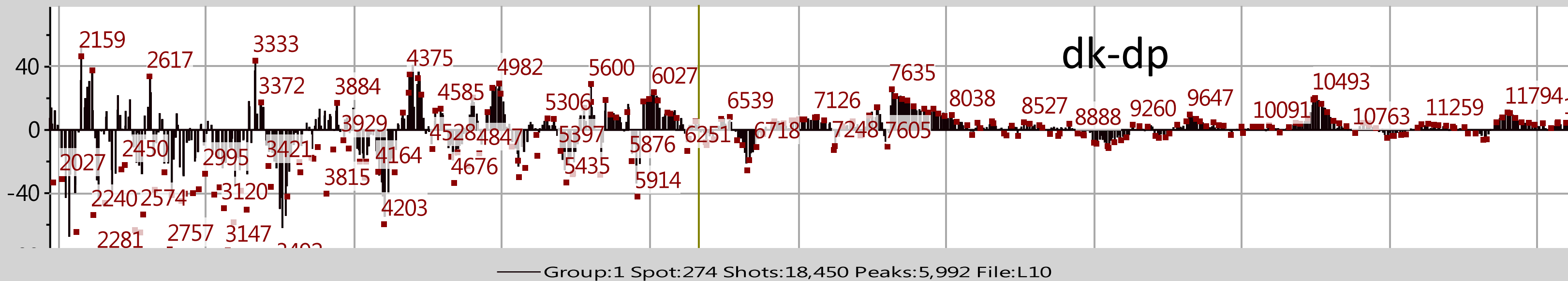


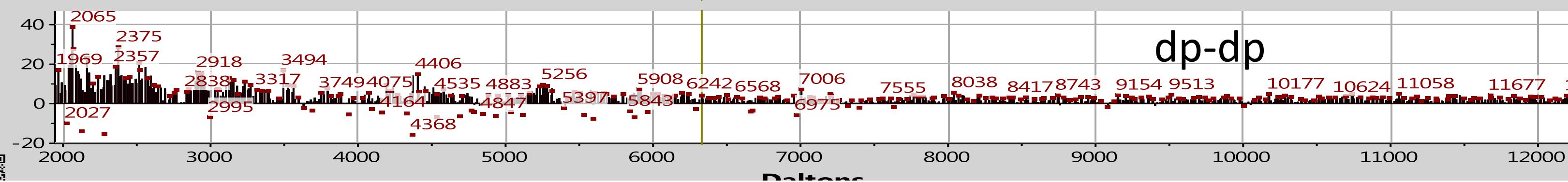
Normalized to Base Peak, 2000 RP

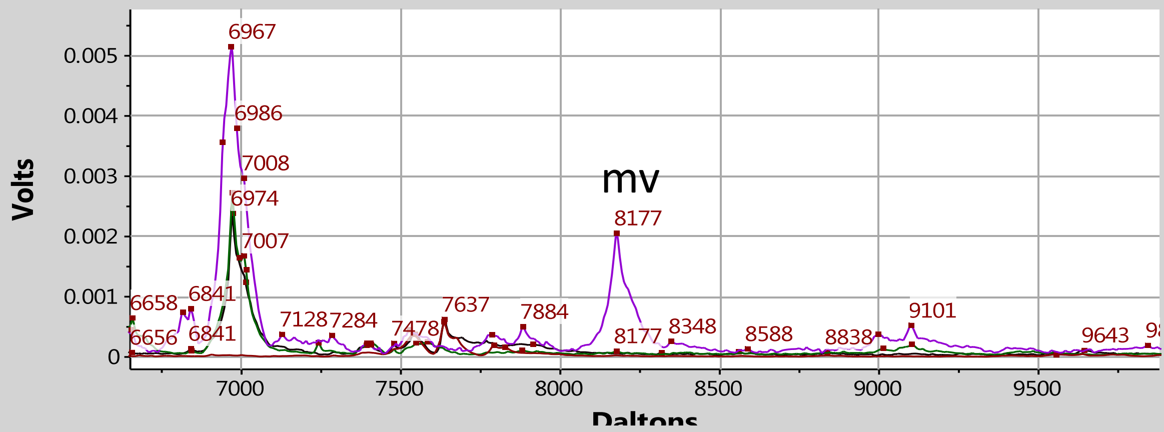
$\Delta N/\sigma$



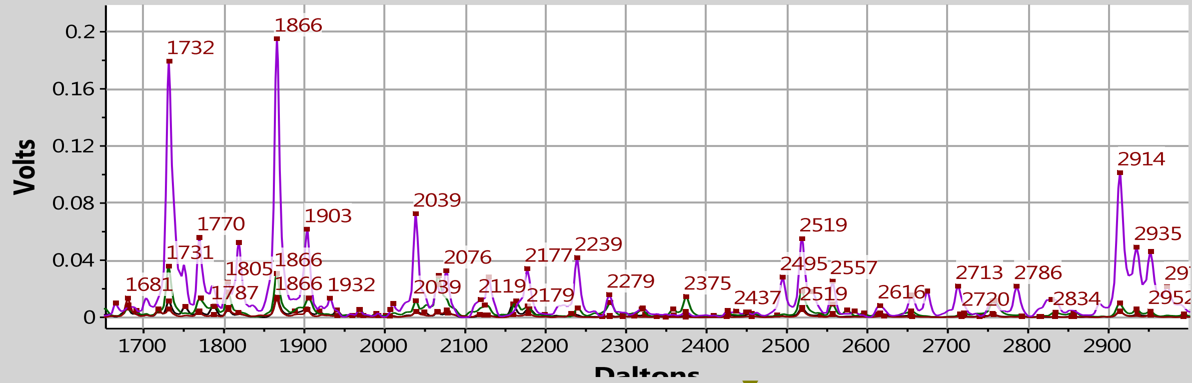




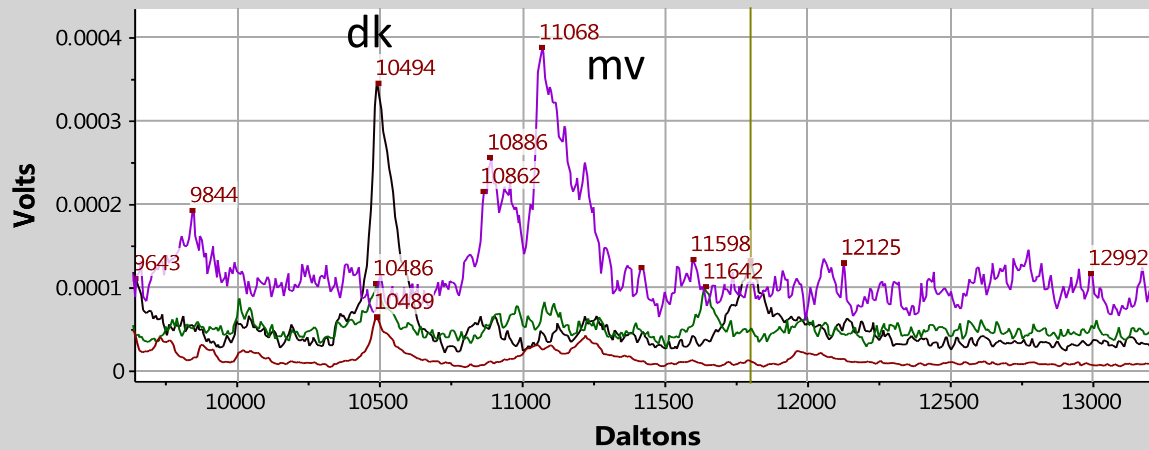




mv
Volts
Daltons

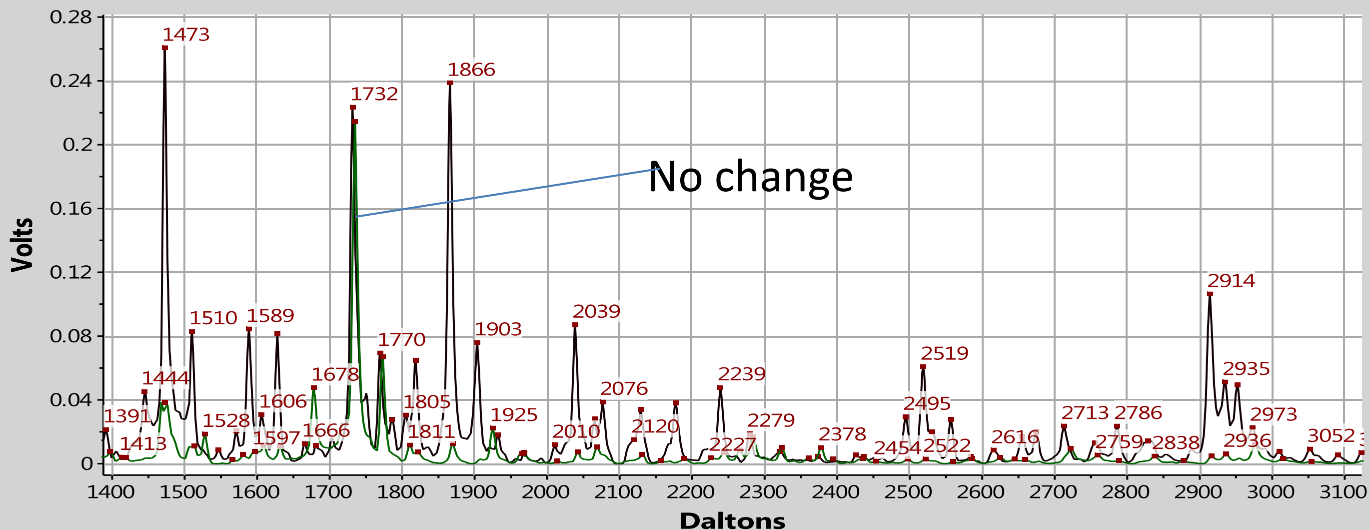


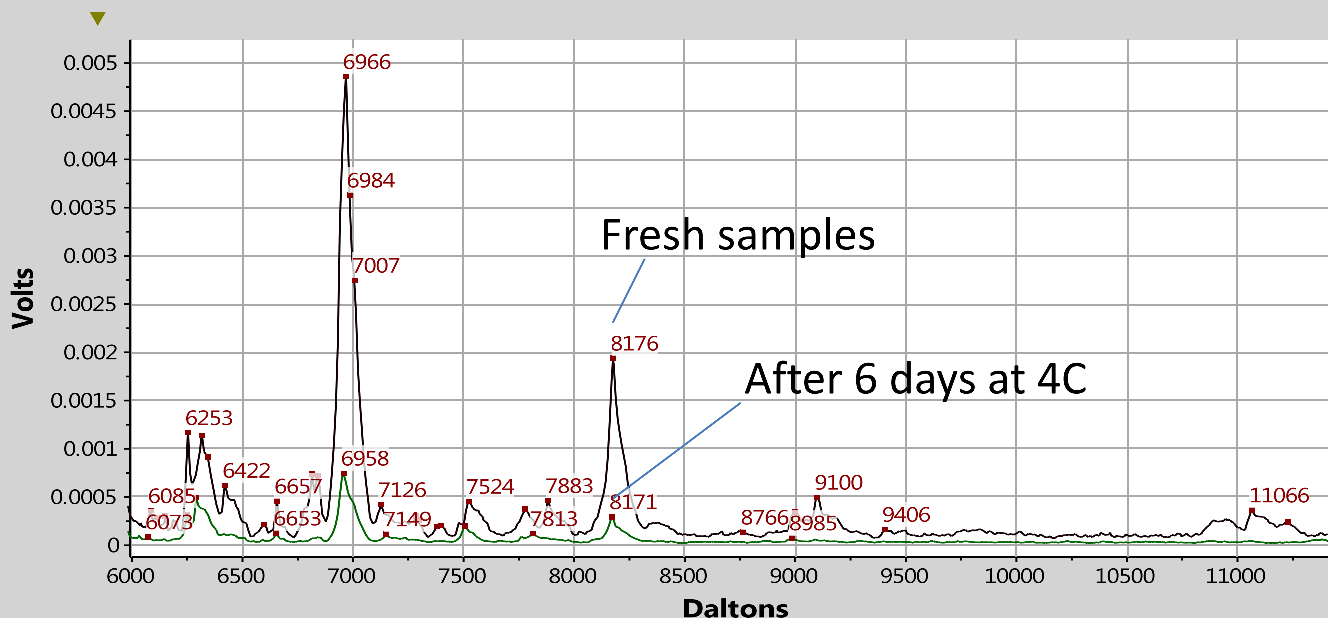
Volts
Daltons



dk
mv
Volts
Daltons

mv

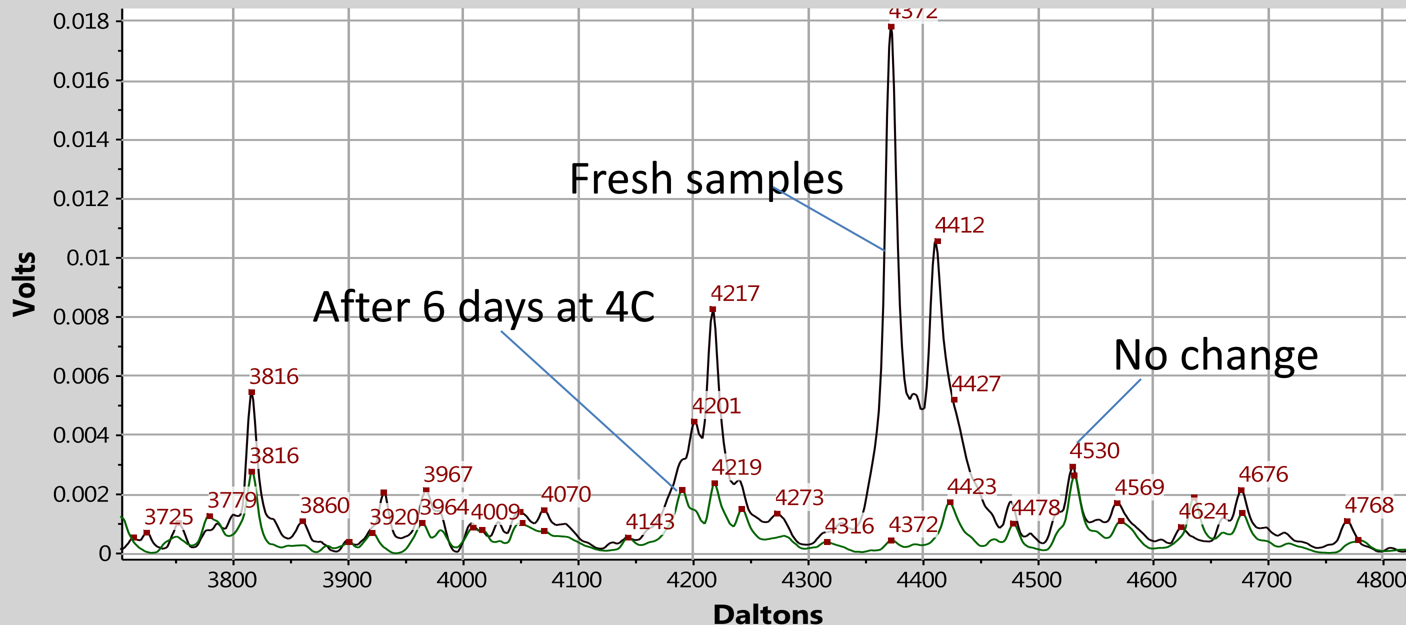




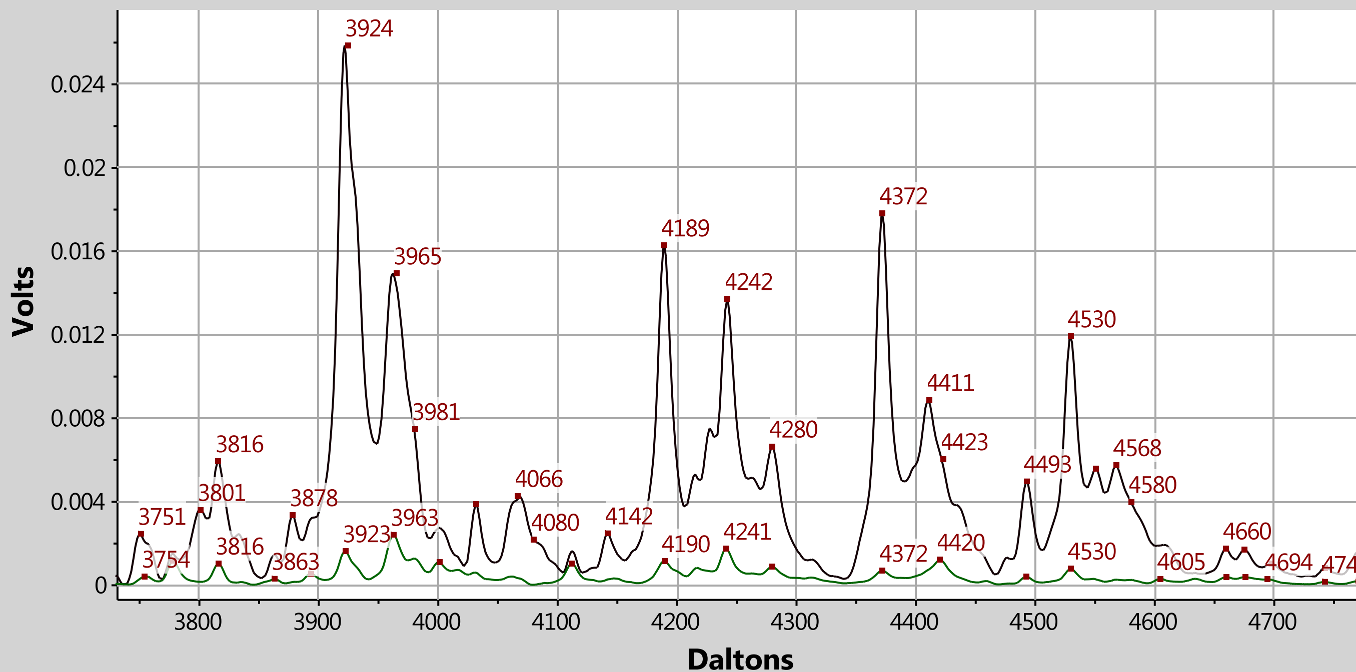
Composite(of 2)#33 ComboBipolar-LinearMS

Group:1 Spot:274 Shots:18,450 Peaks:132 File:L10 Group:1 Spot:322 Shots:18,600 Peaks:136 File:N10

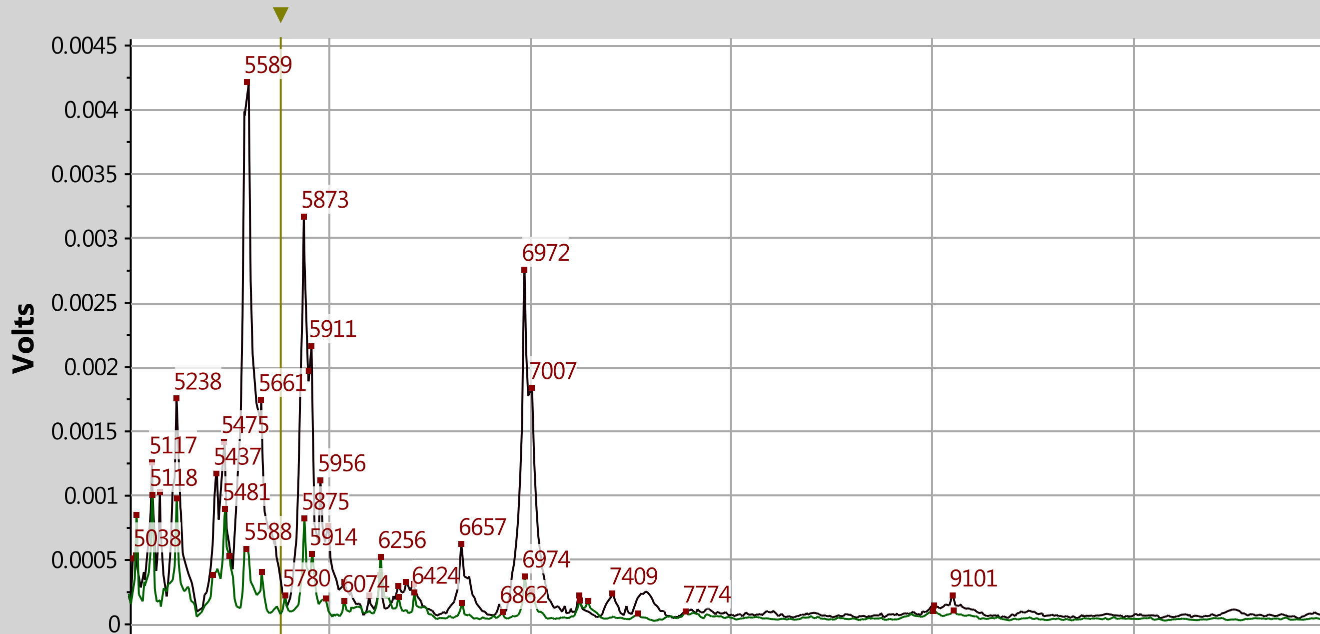
dp



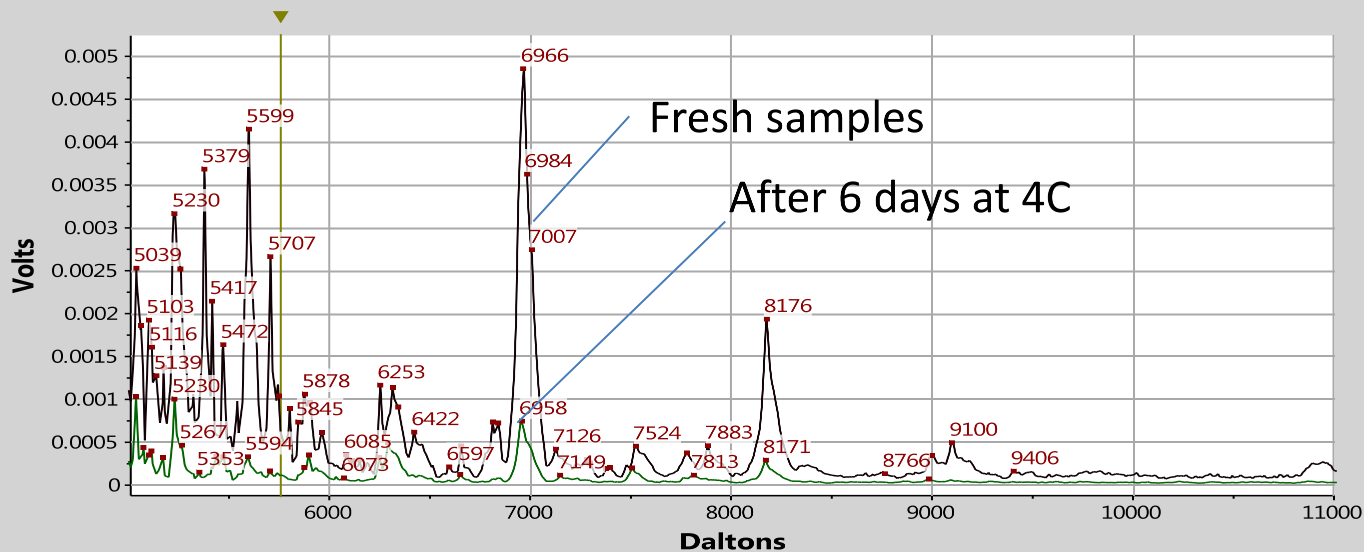
mv



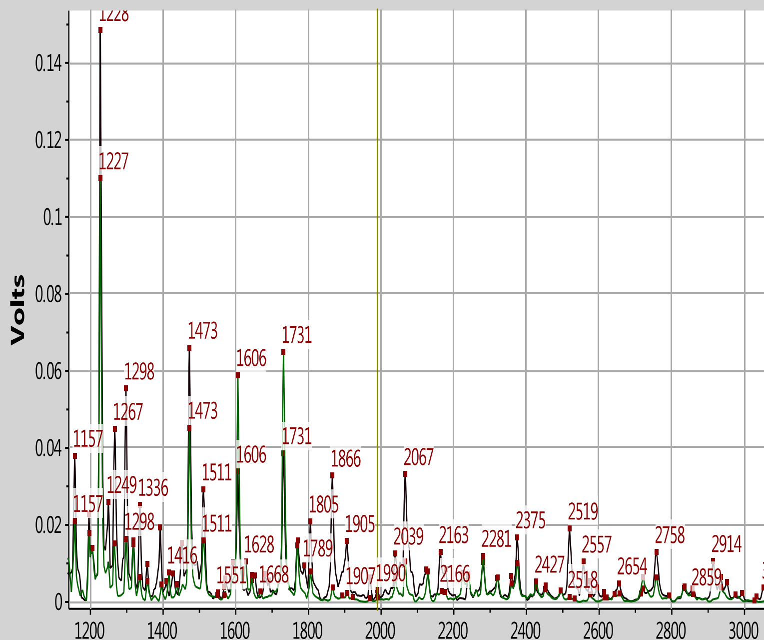
dp

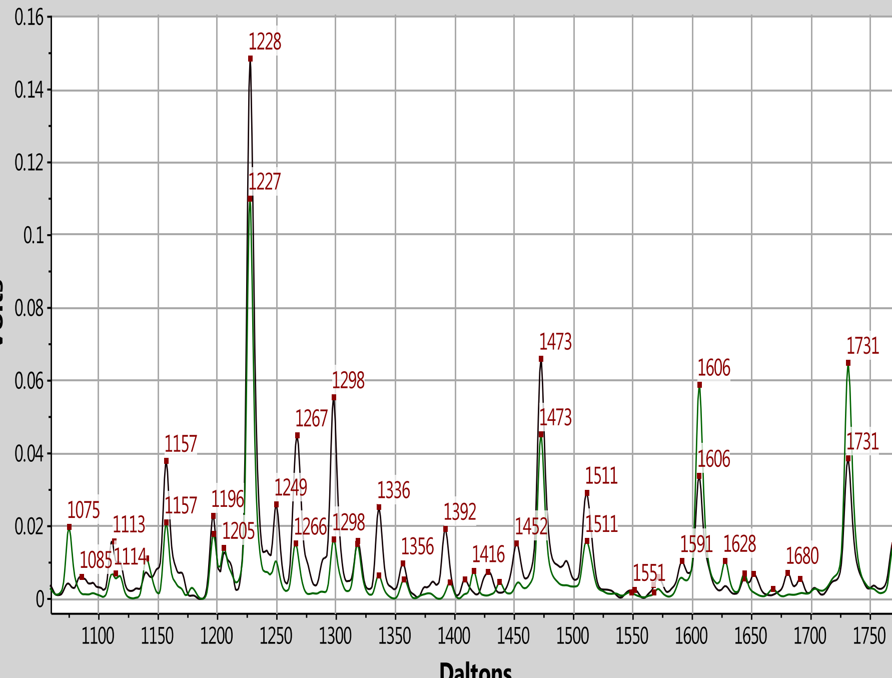


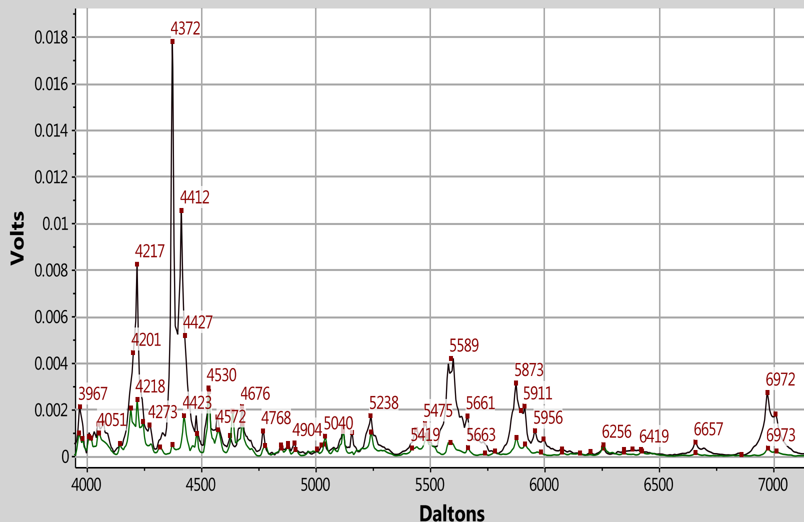
mv

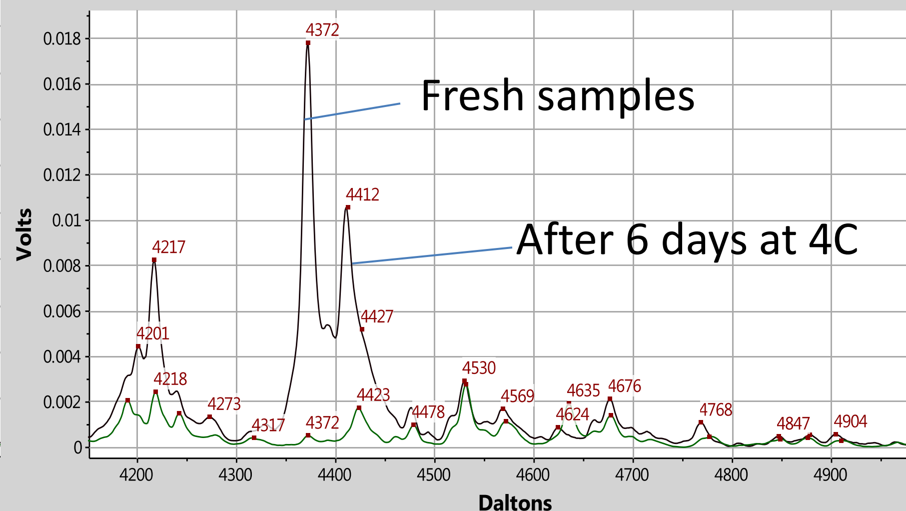


dp









The Future of Diagnostic MS

MALDI-TOF
Very fast (full spectrum/laser shot @5 kHz)
Tradeoff between speed and sensitivity
Can be interfaced with variety of separations
Fully automated, no operator required

Modular BenchTop Systems

SimulTOF Model 100 Linear MALDI
-sensitivity, dynamic range, speed, and simplicity
-wide mass range for proteins, peptides, and small molecules

SimulTOF Model 200 Combo MALDI
-adds high resolution reflector
-accurate mass of peptides and small molecules;

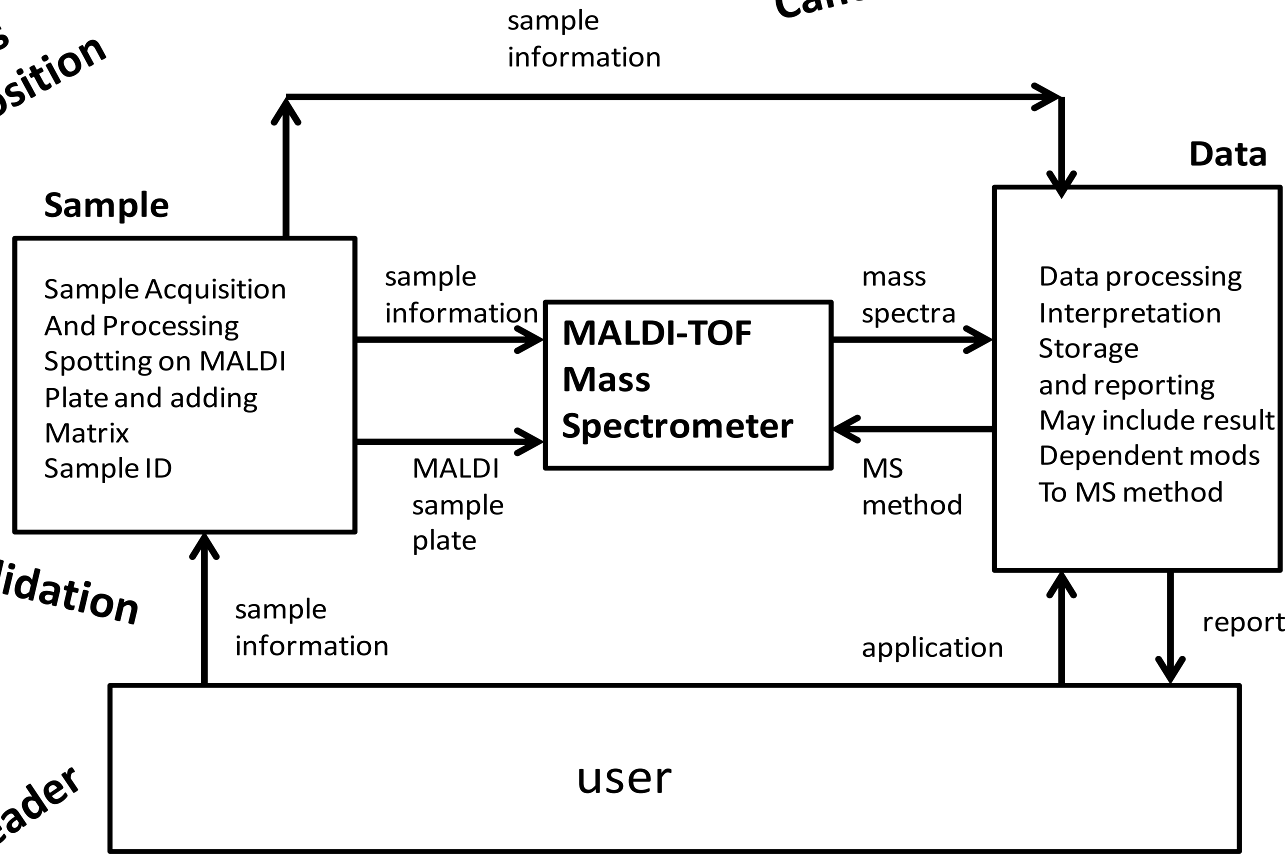
SimulTOF Model 300 MALDI MS-MS
-multiplexed TOF-TOF
-high resolution precursor selection
-identification and quantitation of peptides and small molecules.

Highest Performance at Lowest Cost

Tissue imaging of
Small molecules
for drug disposition

Pathogen Identification

Cancer typing directly from serum,



Biomarker Validation

Protein array reader

Clinical assays of Biomarkers

Tissue imaging of Proteins for
cancer typing

Conclusions

- Resolving power 500-1000 over wide range is routine
- Normalization to TIC or base peak removes most of amplitude variation
- Each spot will yield up to 200,000 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