Development of TOF-MS
from intellectual curiosity to practical technique

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SimulTOF Systems
The Jim Waters Symposium at PittCon
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A Brief History of Time (of flight) with apologies to Stephen Hawking

- 1946 W.E Stephens, *Phys. Rev.* **69**,641
  - "Advances in electronics seem to make practical a type of mass spectrometer in which microsecond pulses of ions are selected every millisecond from an ordinary low-voltage ion source. In travelling down the vacuum tube, ions of different M/e have different velocities and consequently separate into groups spread out in space. … This type of mass spectrometer should offer many advantages over present types. The response time should be limited only by the repetition rate (milliseconds)… Magnets and stabilization equipment would be eliminated. Resolution would not be limited by smallness of slits or alignment. Such a mass spectrometer should be well suited for composition control, rapid analysis, and portable use."

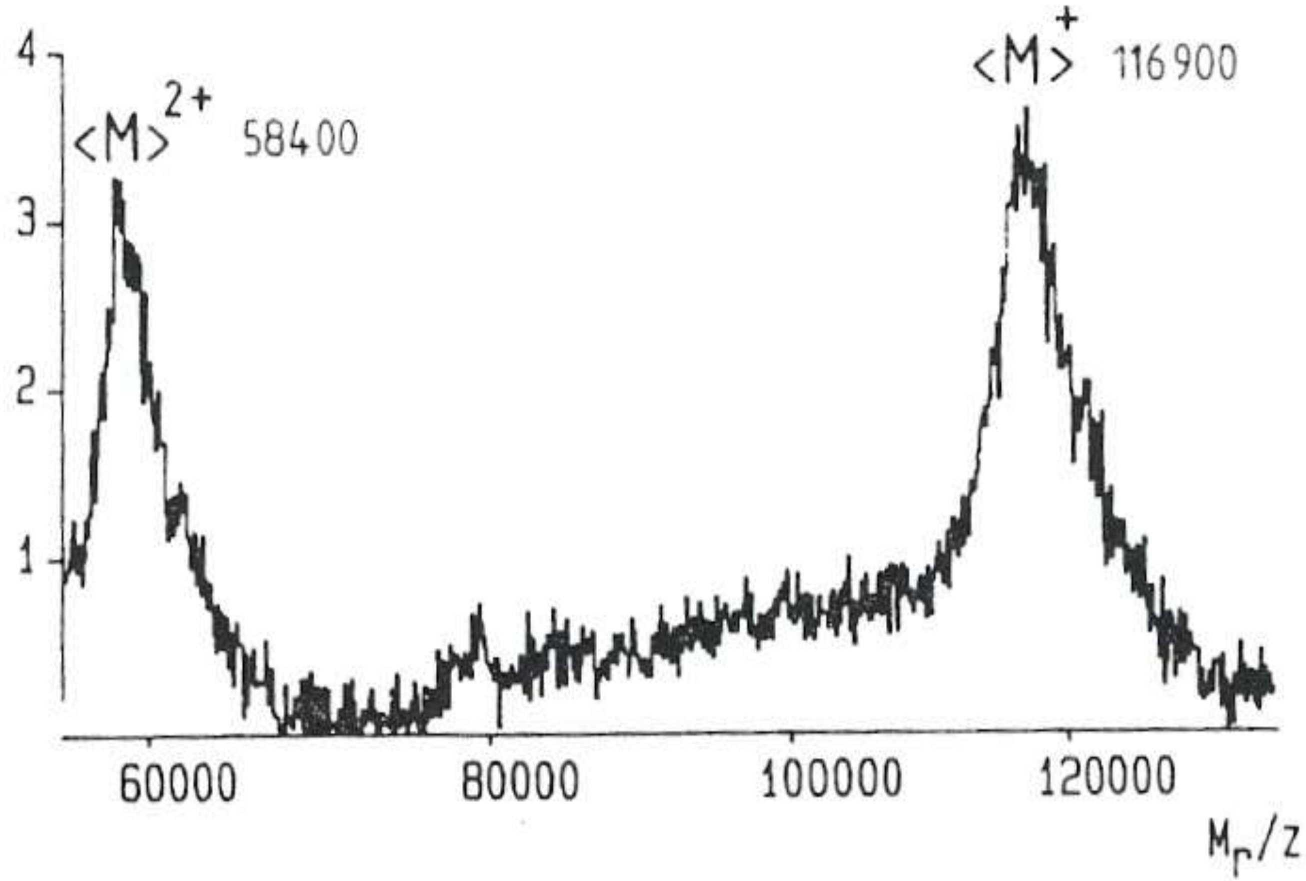
Brief History of Time (of flight)

- 1948 Cameron & Eggers, *Rev. Sci. Instr*. **19**, 605.
  - First working TOF
- 1953 **Wiley & McLaren, *Rev. Sci. Instr*. 26, 1150**
  - **First practical TOF. Energy & Time Lag Focusing.**
- 1959 Gohlke, *Anal. Chem*. **31**, 535
  - GC-TOF
- 1963 Vestal & Wharhaftig, ASMS, 358.
  - Coincidence TOF, first ion counting TDC
- 1973 **Mamyrin et al, *Sov. Phys. JETP* 37, 45.**
  - **Reflectron, higher resolution**
- 1974 Macfarlane et al, *Biochem. Biophys. Res. Comm*. **60**, 616
  - ^{252}Cf Plasma desorption. Proteins Fly!!!!

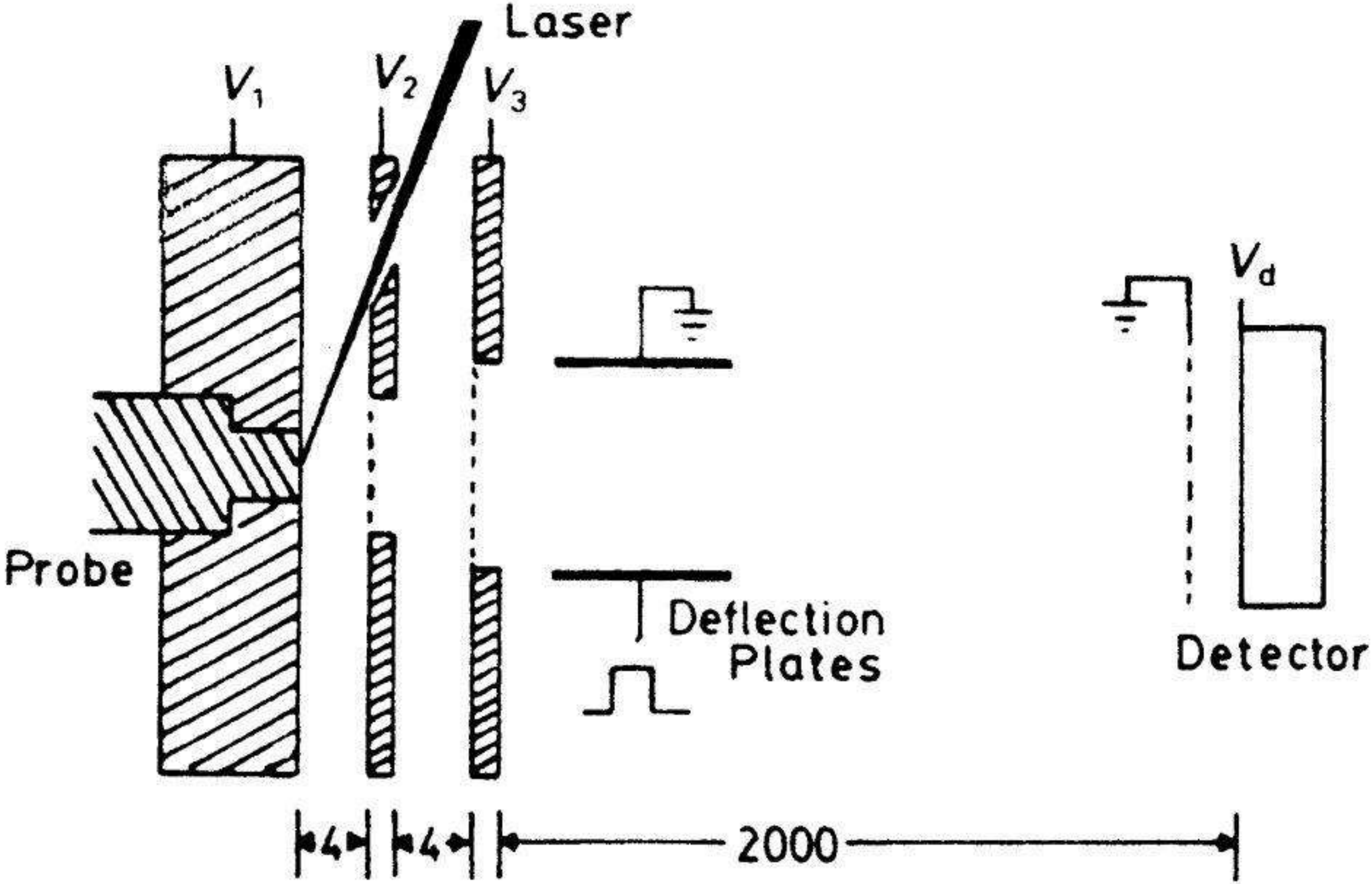
Brief History of Time (of flight)

- 1988 Karas & Hillenkamp, *Anal. Chem.* **60**, 2299.
  - MALDI - Really big proteins fly!!!
- 1991 Dodenov et al, 12th Int. MS Conf.
  - O-TOF - Electrospray works with TOF
- 1993 Kaufman, Spengler & Lutzenkirchen, RCM 7, 902.
  - Post-source decay MALDI
- 1994 Brown & Lennon, Sunriver, p63.
  - Delayed extraction MALDI - Makes MALDI-TOF routine
- 1996 Morris et al, *RCM* **10**, 889.
  - Q-TOF
- 2001
  - TOF-TOF

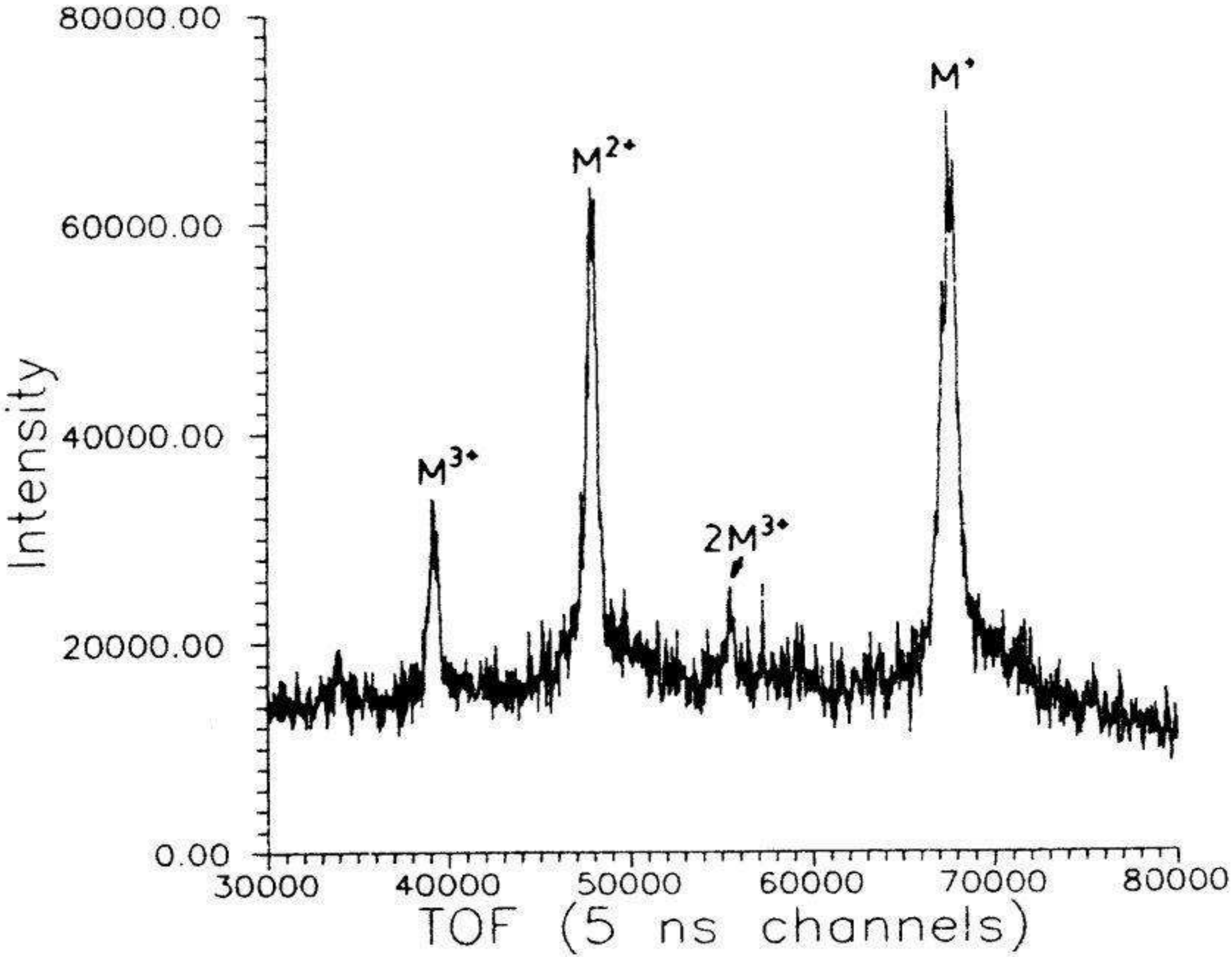
Early History of MALDI-TOF



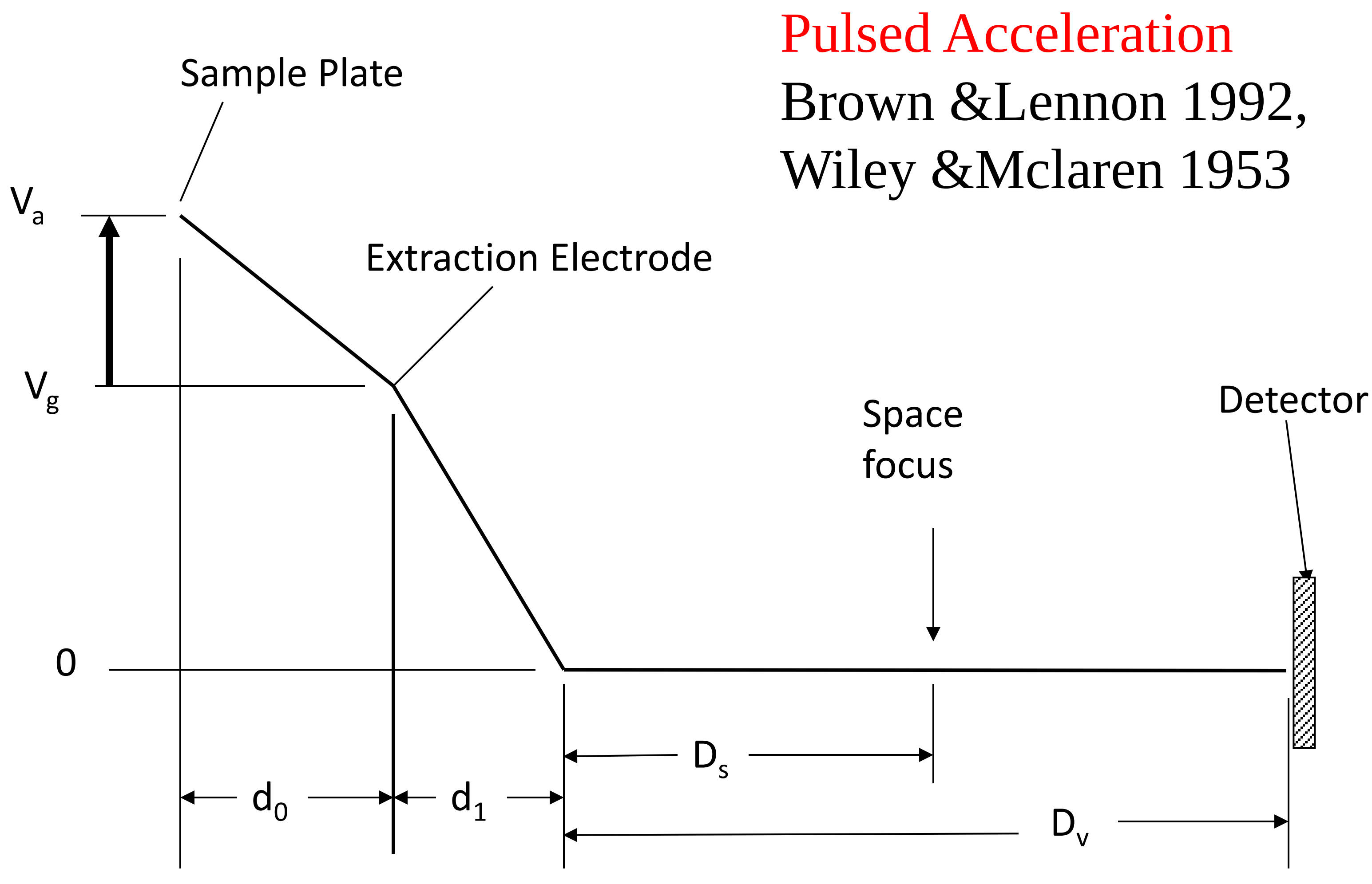
Karras &Hillenkamp 1988



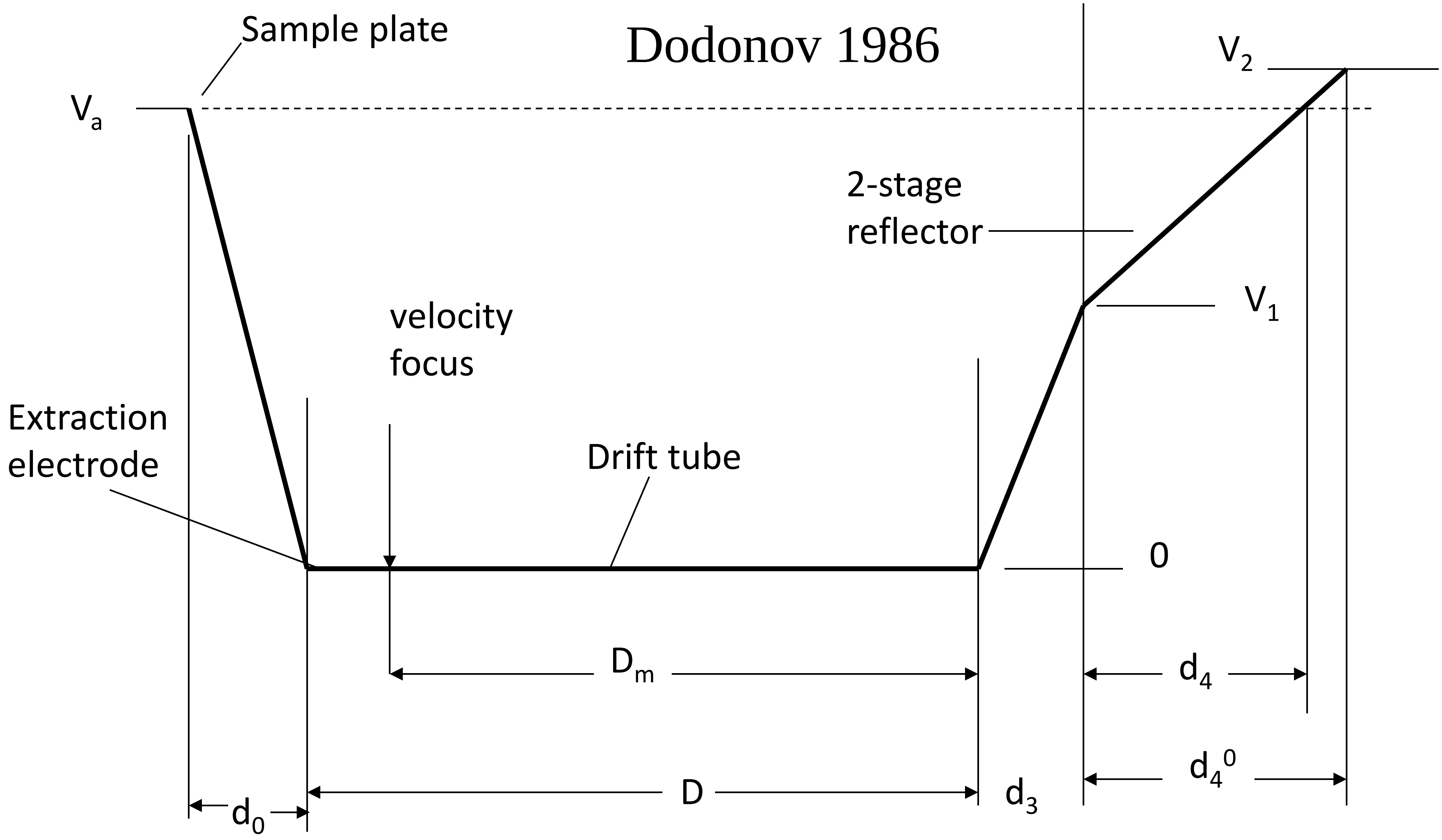
Beavis &Chait 1989



Beavis &Chait 1989



Initial velocity of ions from MALDI is high and independent of mass. This is the major contribution to time dispersion in static MALDI.



Delayed extraction source plus 2-stage reflector
Makes high resolution MALDI practical

Table 1. Effective flight paths for the mass analyzers employed in this work

Analyzer	Linear (m)	Reflector (m)
RP	1.3	2.0
EL	2.0	3.0
XL	4.2	6.6

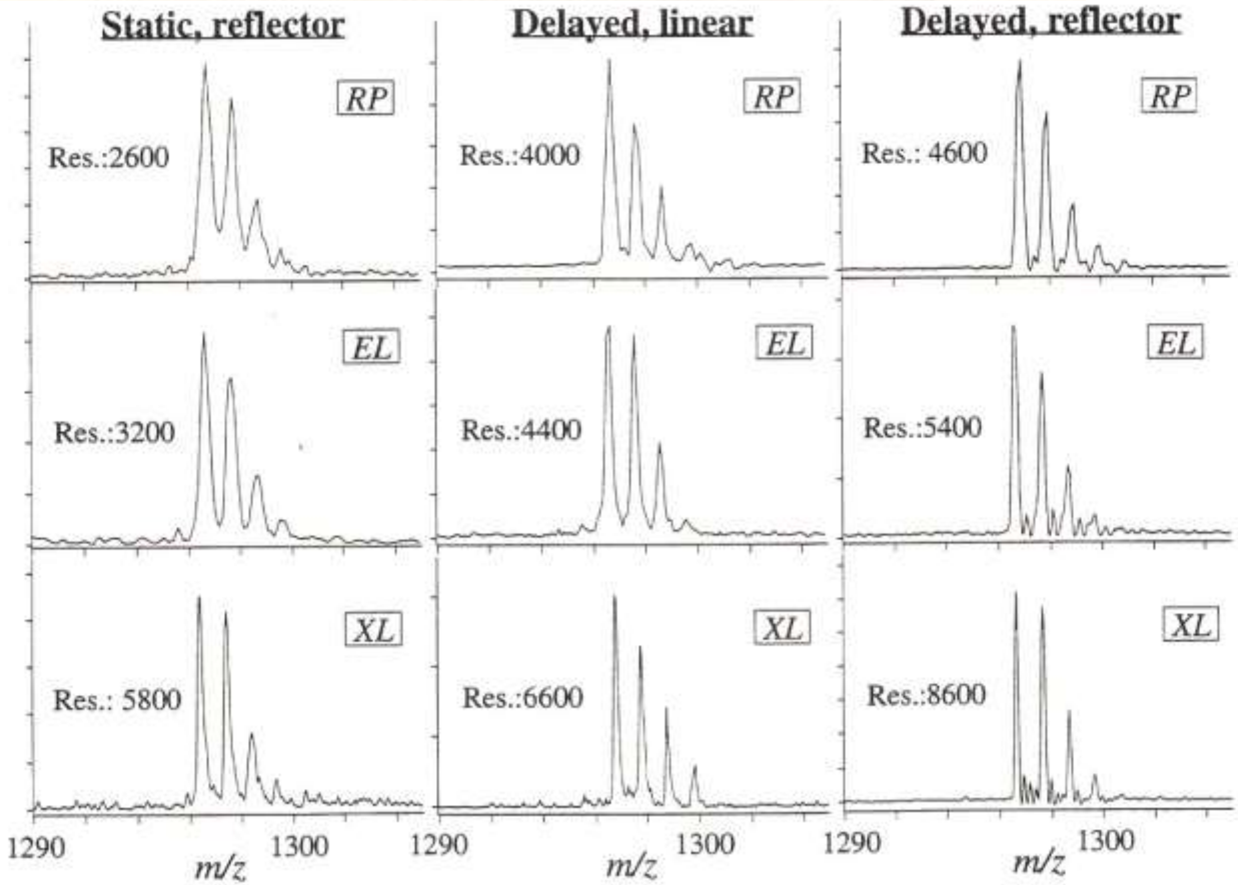


Figure 3. Comparison of the resolution and signal-to-noise (S/N) ratio obtained for the molecular ion region of angiotensin I (MH^+, monoisotopic $m/z = 1296.68$) in three different operating modes (static linear, delayed extraction linear and delayed extraction reflector) as a function of the analyzer geometry (RP, EL, XL) as given in Table 1. Matrix: α-cyano-4-hydroxycinnamic acid.

Contributions to relative peak width, $\Delta m/m$

Initial position, δx: $R_{s1} = [(D_v - D_s)/D_e](\delta x/d_0 y)$

Initial velocity, δv_0: $R_{v1} = (4d_0 y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

$R_{v2} = 2[2d_0 y/(D_v - D_s)]^2 (\delta v_0/v_n)^2$

$R_{v3} = 2[2d_0 y/(D_v - D_s)]^3 (\delta v_0/v_n)^3$

Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

Trajectory error, δL: $R_L = 2\delta L/D_e$

Not small!!!

Voltage error, δV: $R_V = \delta V/V$

Resolving power:

$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$

Contributions to relative peak width, $\Delta m/m$, with 1st and 2nd order velocity focusing (size matters)

Initial position, δx: $R_{s1} = [2/\mathbf{K}](\delta x/D_e)$

Initial velocity, δv_0: $R_{v1} = (4 d_0 y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

$R_{v2} = 2[\mathbf{K}]^2 (\delta v_0/v_n)^2 = 0$

$R_{v3} = 2[\mathbf{K}]^3 (\delta v_0/v_n)^3$

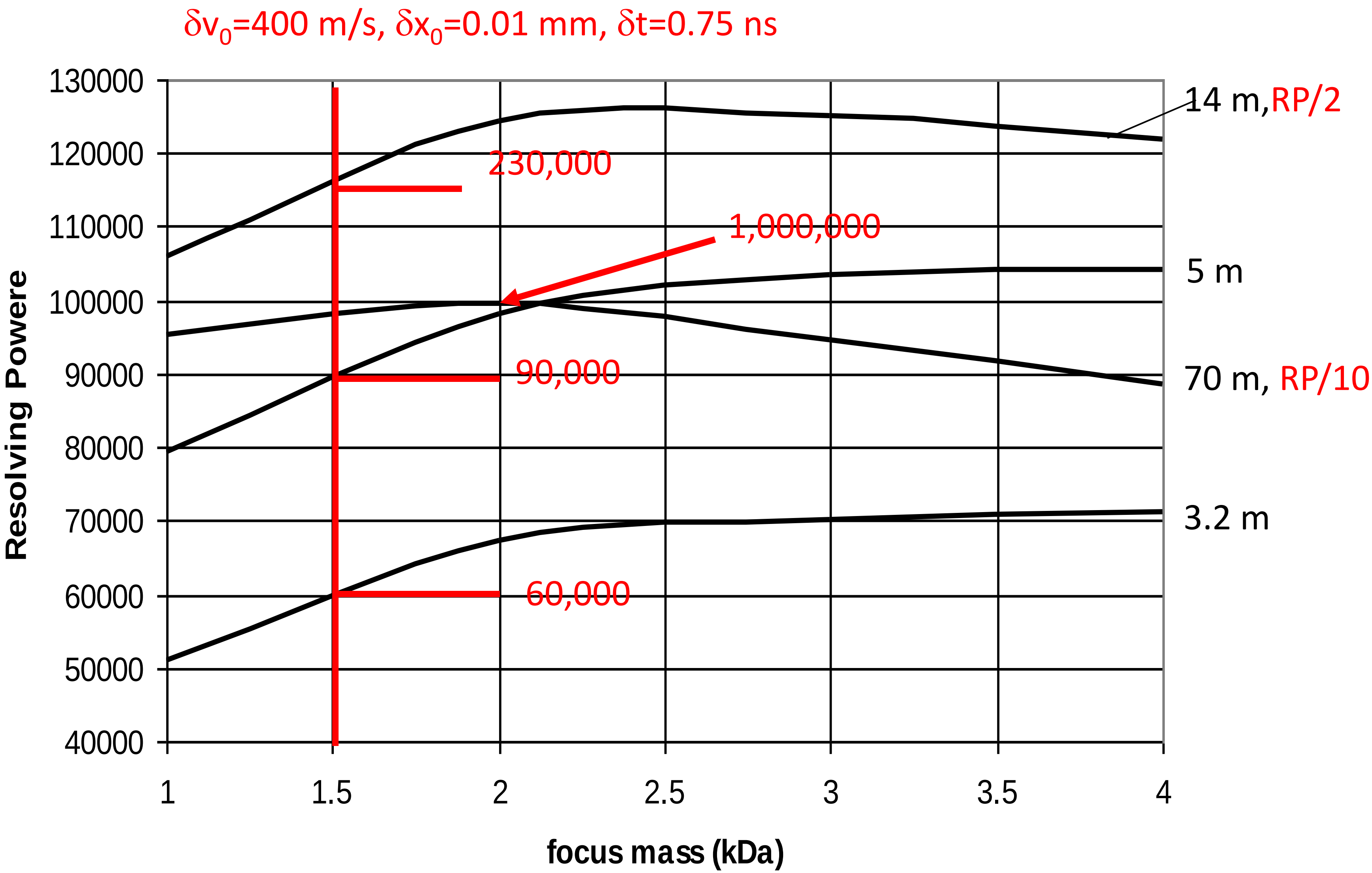
Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

Trajectory error, δL: $R_L = 2\delta L/D_e$

Voltage error, δV: $R_V = \delta V/V$

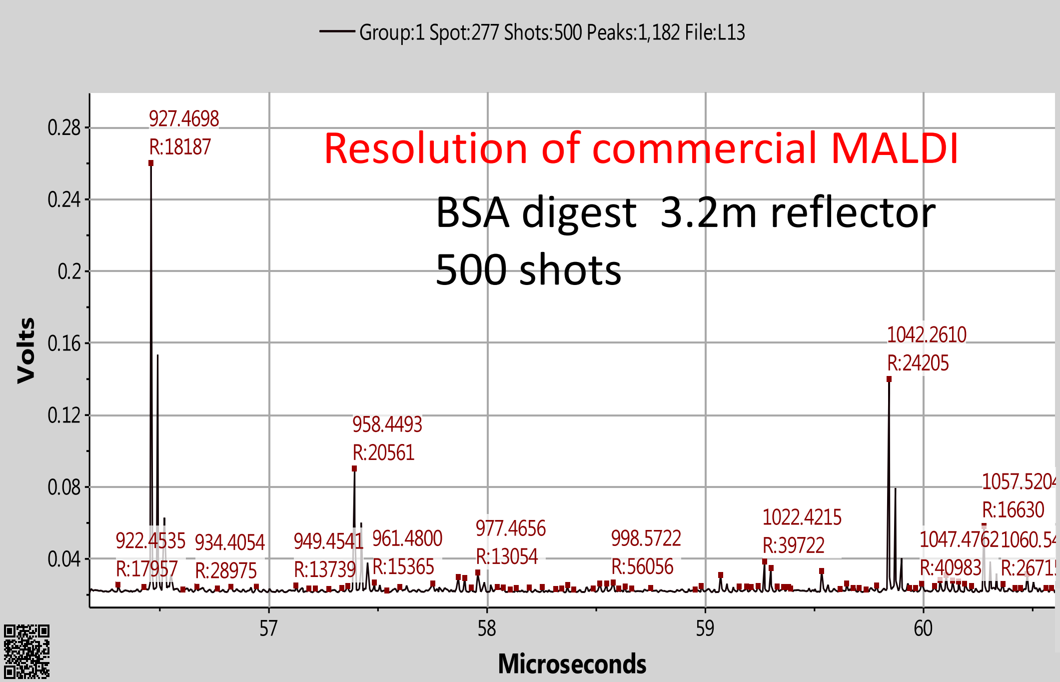
Resolving power:

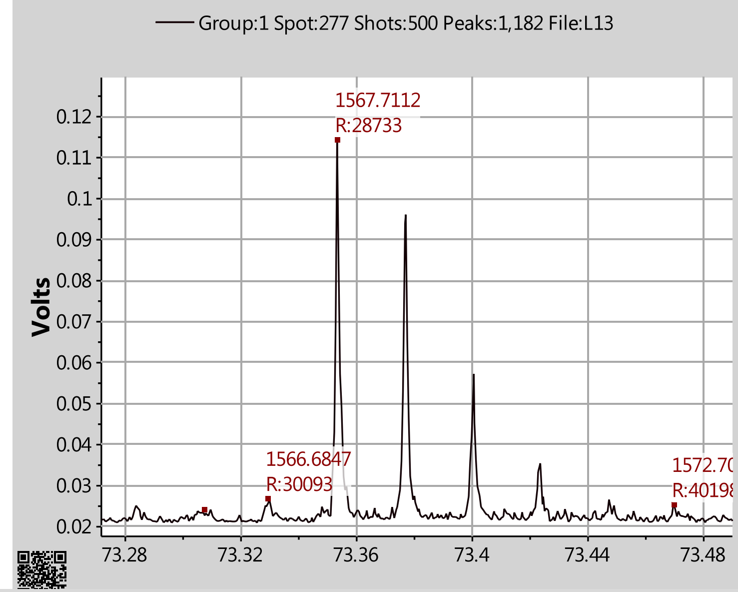
$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$

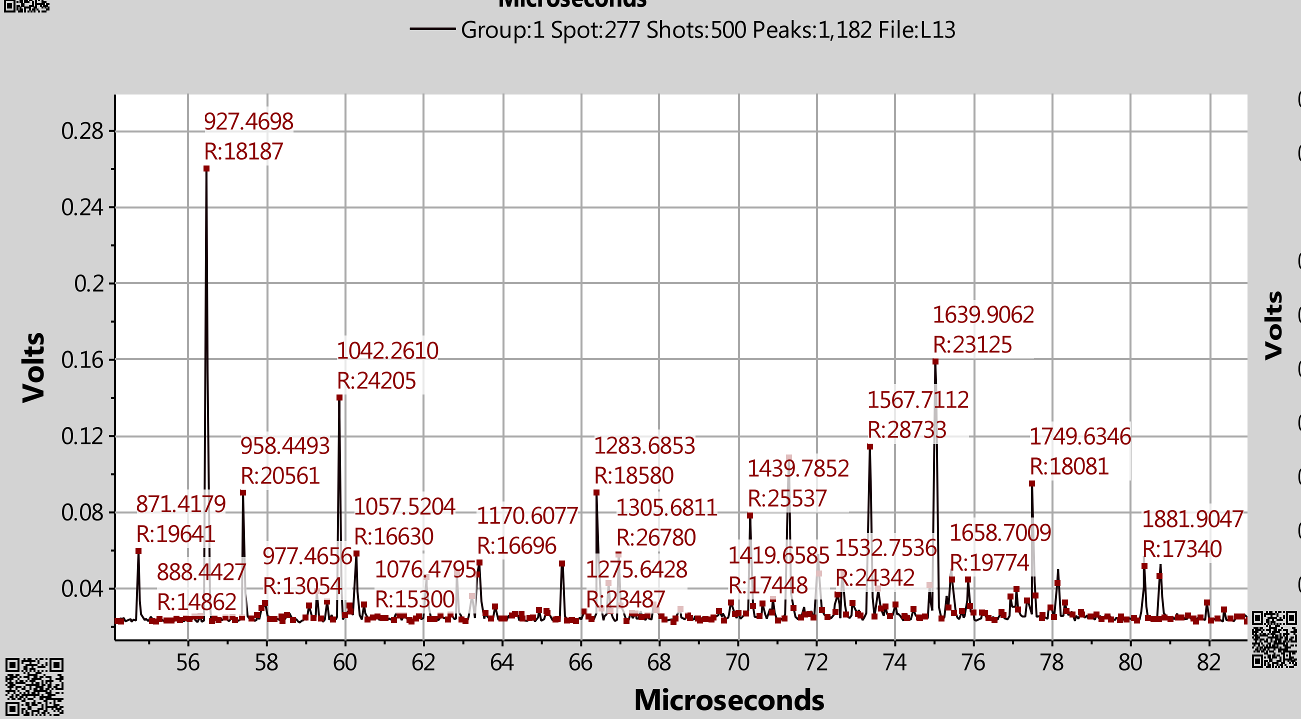


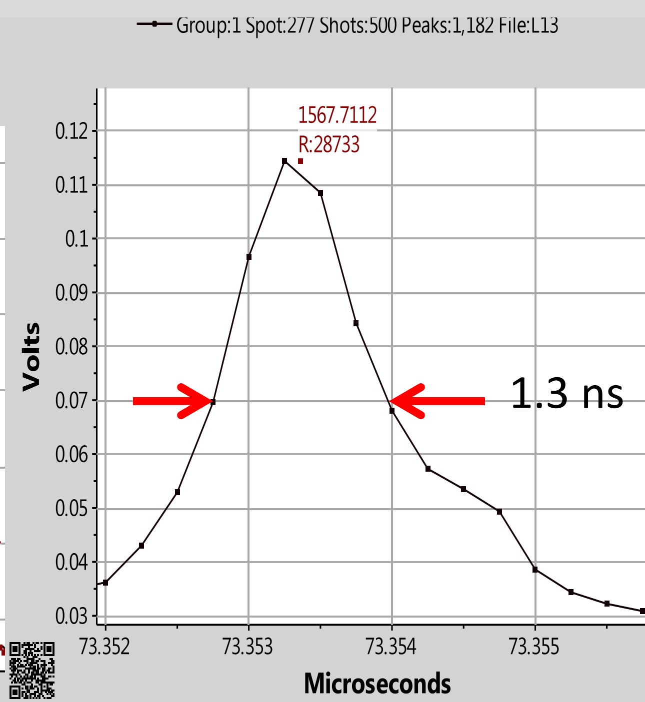
Maximum resolving power at focus mass under optimized conditions.

Does not include contributions from trajectory error, HV noise, and collisions


Resolution of commercial MALDI
BSA digest 3.2m reflector
500 shots
Group:1 Spot:277 Shots:500 Peaks:1,182 File:L13
927.4698 R:18187
958.4493 R:20561
1042.2610 R:24205
1057.5204 R:16630
922.4535 R:17957
934.4054 R:28975
949.4541 R:13739
961.4800 R:15365
977.4656 R:13054
998.5722 R:56056
1022.4215 R:39722
1047.4762 R:40983
Volts
Microseconds

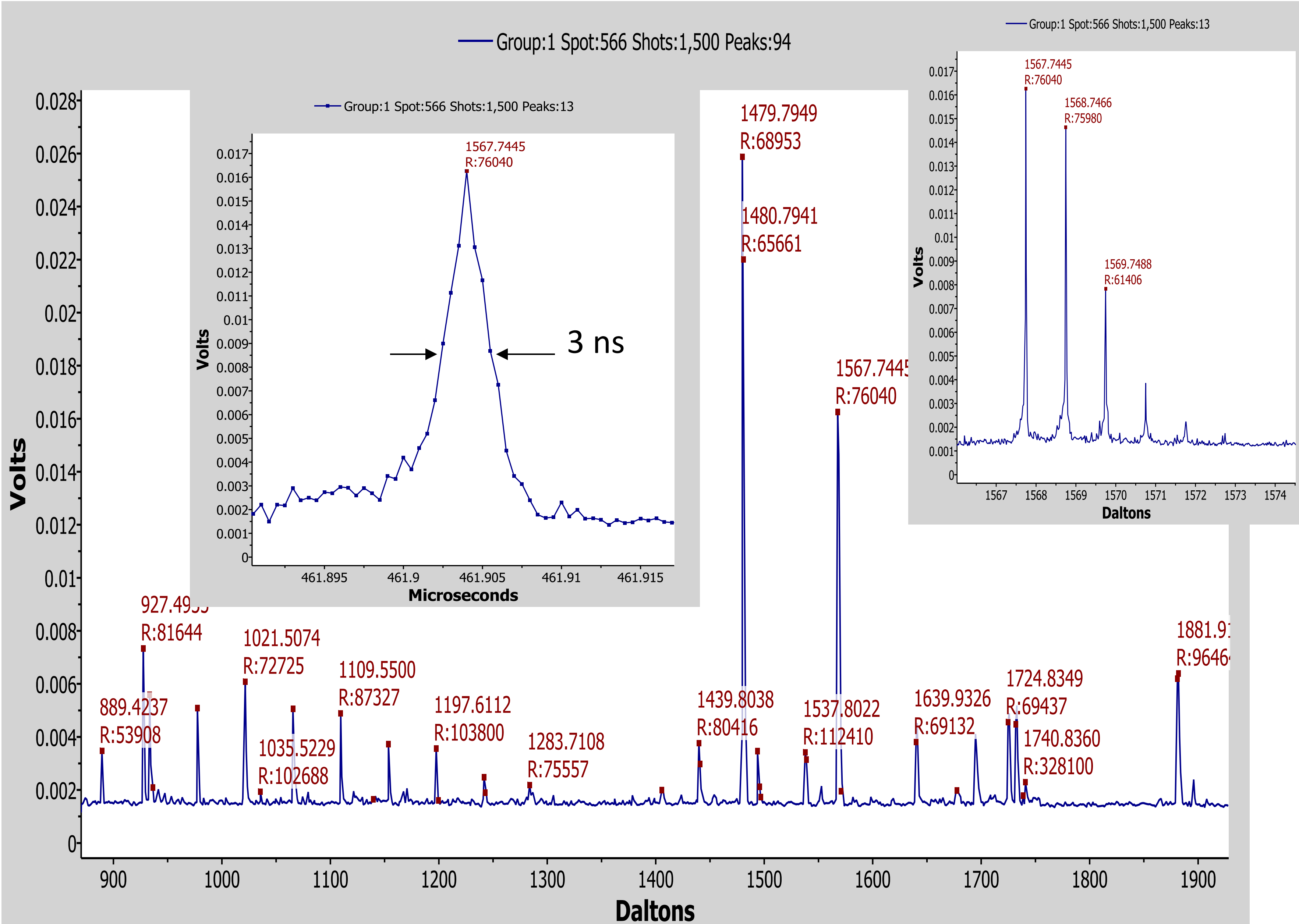

Group:1 Spot:277 Shots:500 Peaks:1,182 File:L13
1567.7112 R:28733
1566.6847 R:30093
Volts


Group:1 Spot:277 Shots:500 Peaks:1,182 File:L13
927.4698 R:18187
1042.2610 R:24205
958.4493 R:20561
871.4179 R:19641
888.4427 R:14862
977.4656 R:13054
1057.5204 R:16630
1076.4795 R:15300
1170.6077 R:16696
1283.6853 R:18580
1305.6811 R:26780
1275.6428 R:23487
1439.7852 R:25537
1419.6585 R:17448
1532.7536 R:24342
1567.7112 R:28733
1639.9062 R:23125
1658.7009 R:19774
1749.6346 R:18081
1881.9047 R:17340
Volts
Microseconds


Group:1 Spot:277 Shots:500 Peaks:1,182 File:L13
1567.7112 R:28733
1.3 ns
Volts
Microseconds

6 m single mirror
14 m effective

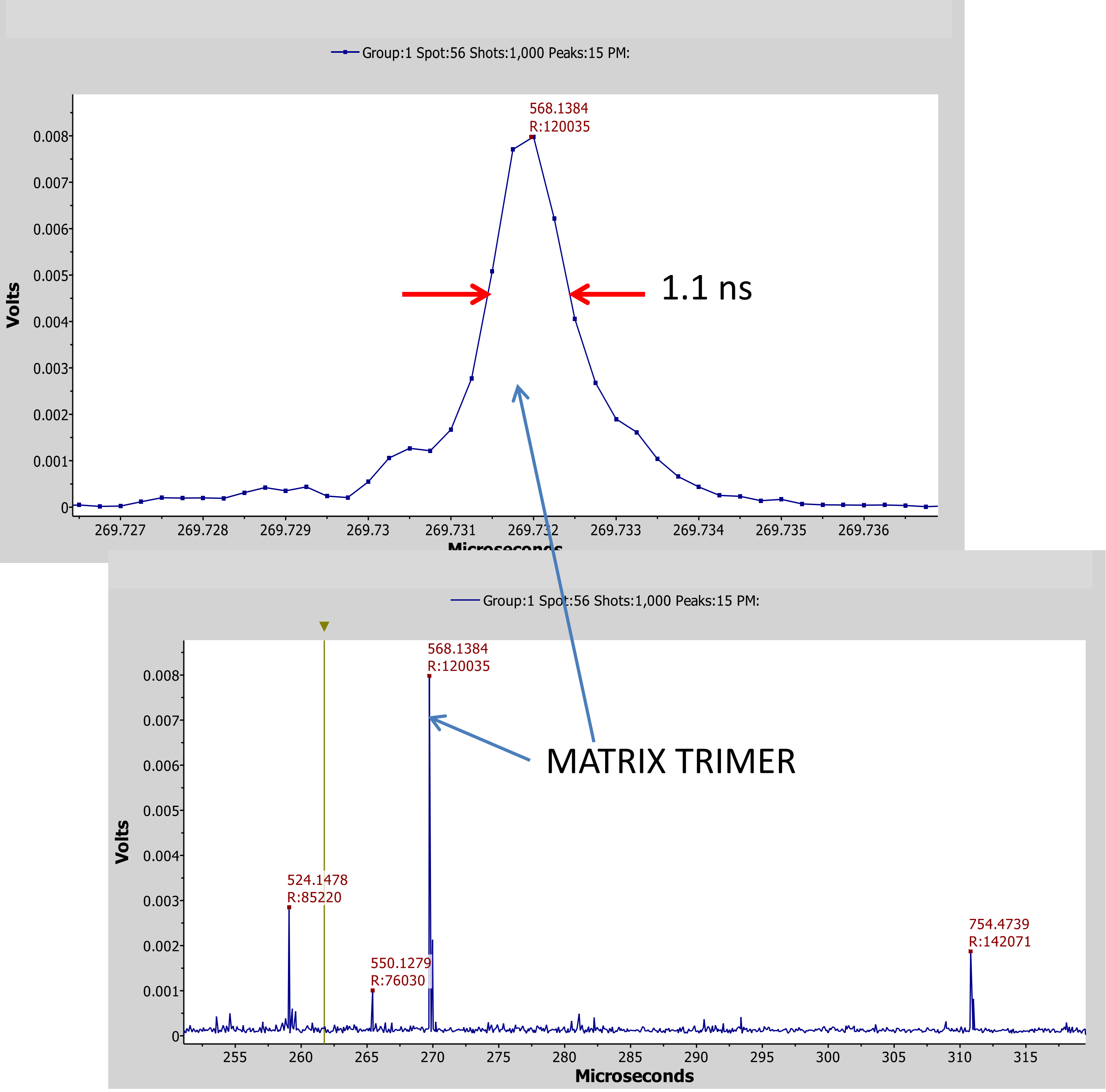
Source is on first floor and mirror on second floor



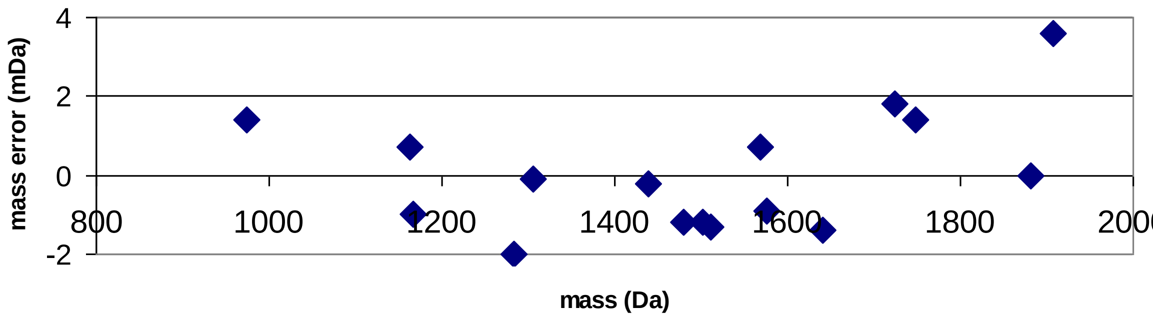
14m single reflector BSA digest

14m
Single
Reflector

Highest
Resolving
Power
Recorded
For
MALDI-TOF

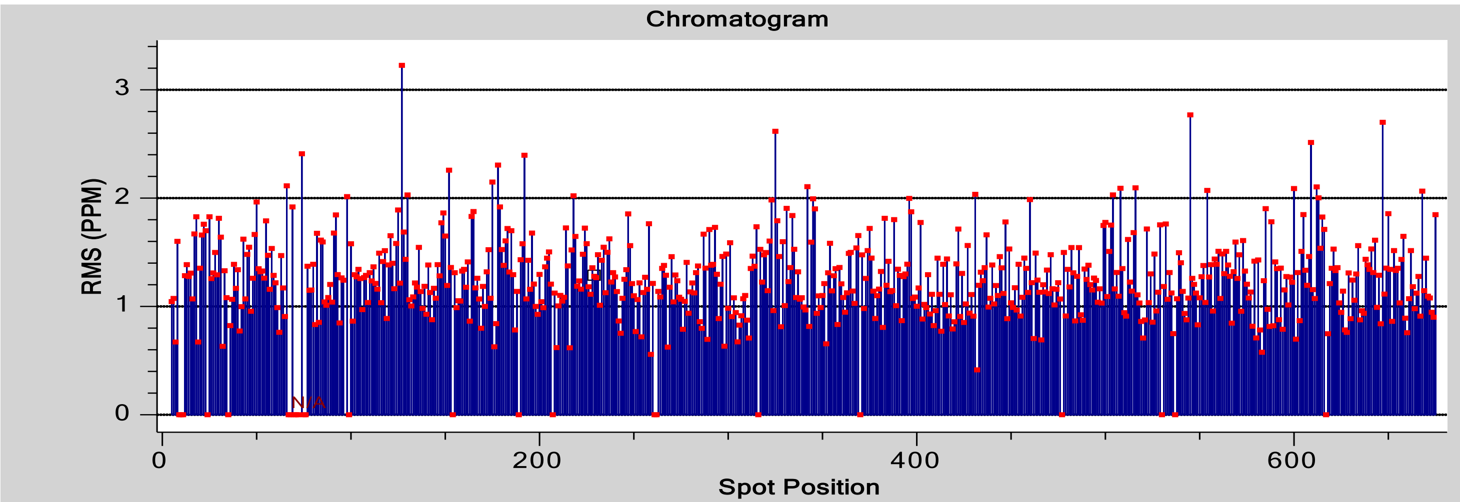


Calibration Equation: $m^{1/2}=D_0 + D_1 t[1+D_2 t+D_3 t^2]$
Determine coefficients by least square fit to multiple Peaks covering broad mass range

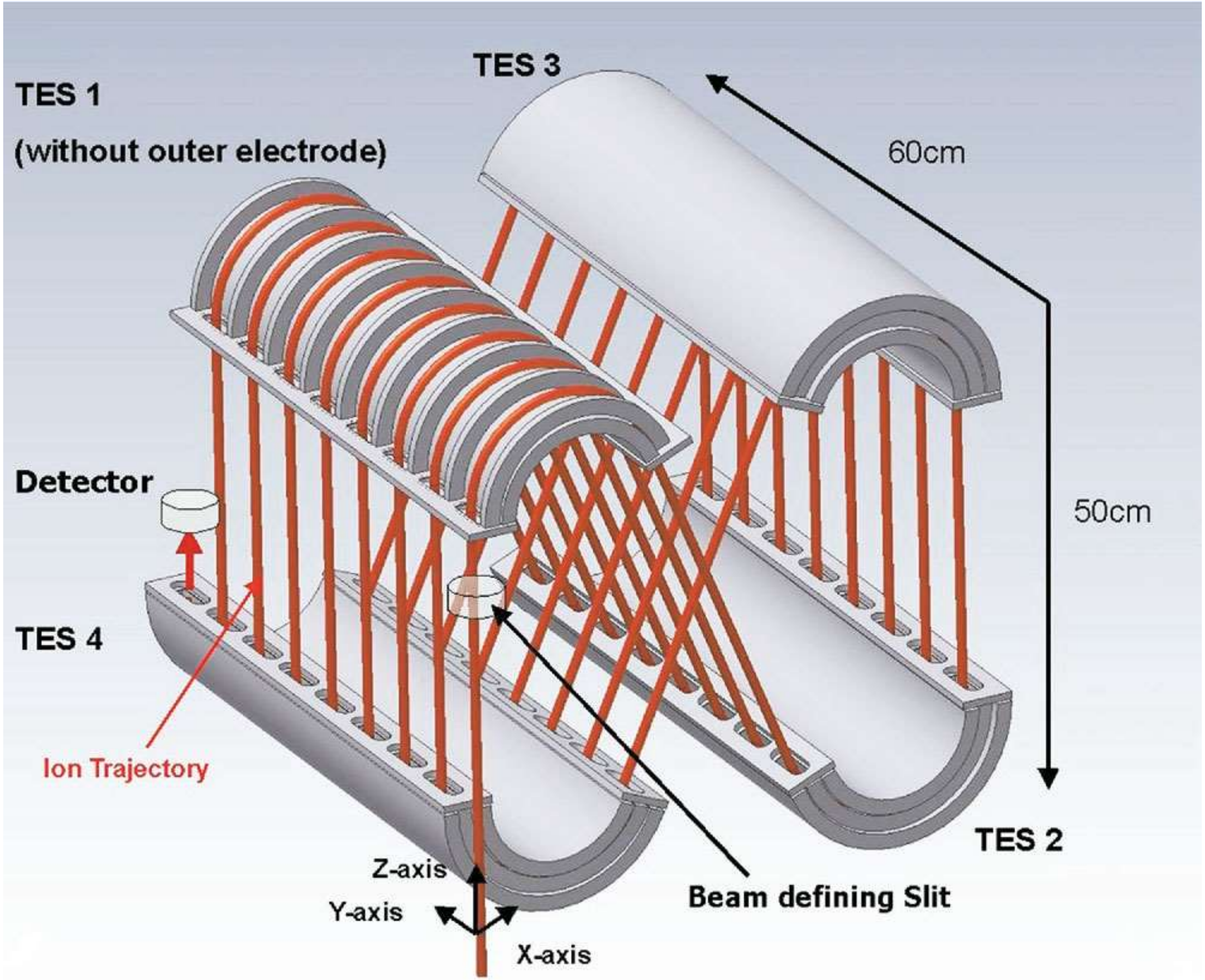


Average=-0.1 mDa

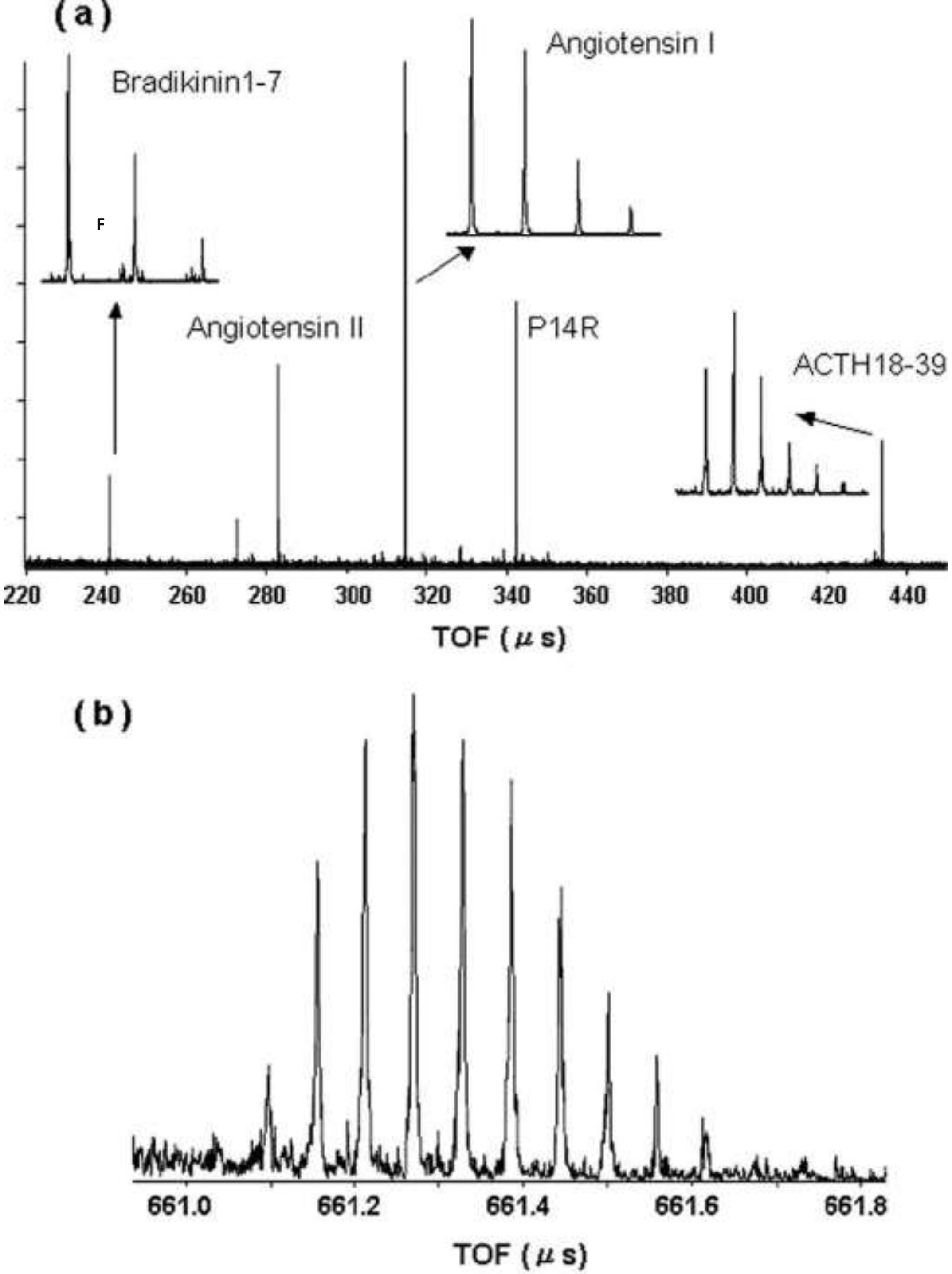
RMS =0.99 ppm

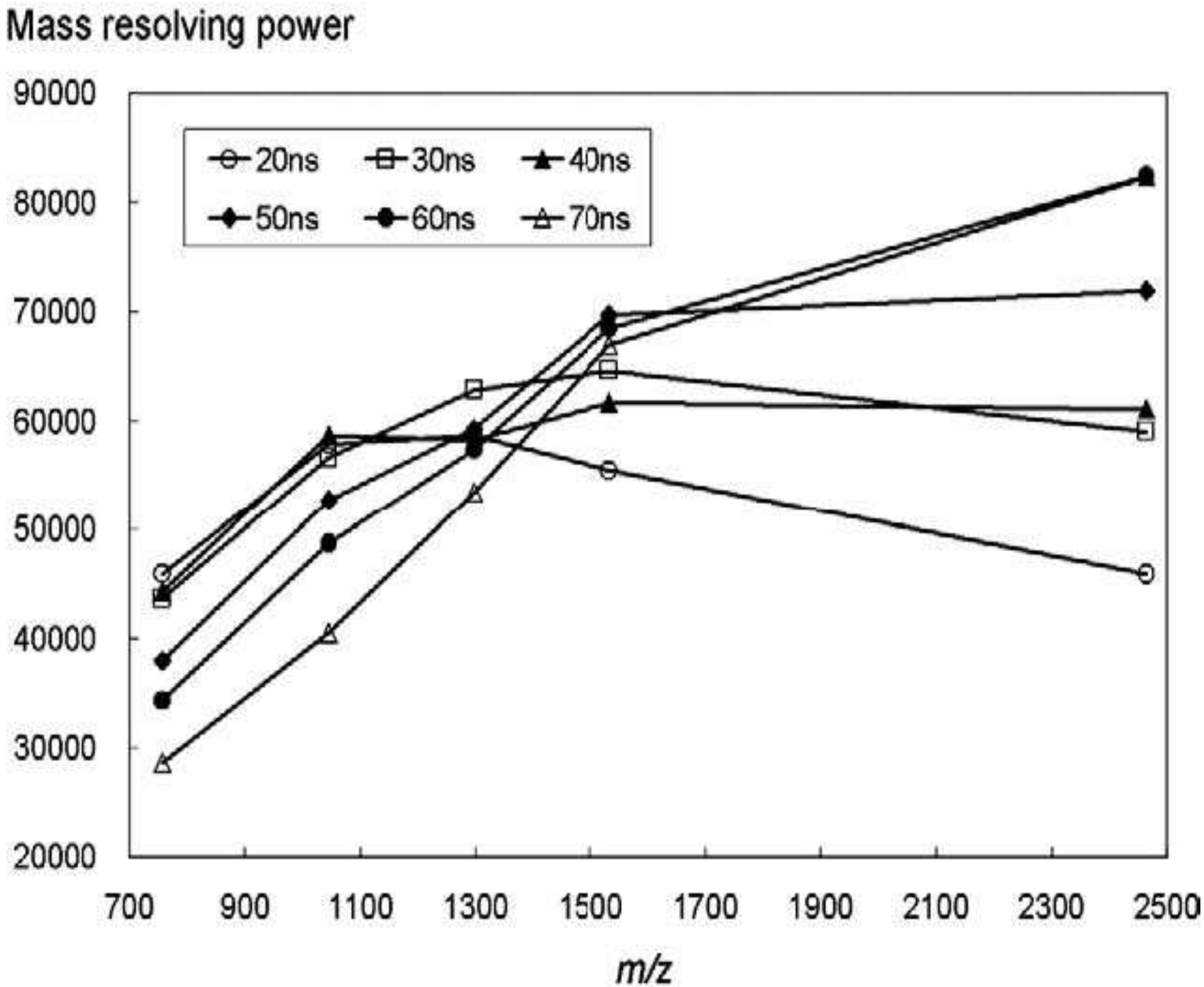


RMS error for 10 peaks in spectrum of tryptic digest of BSA for all 675 spots on a 102x108 mm sample plate with automatic 2-point internal Calibration for 3.2 m analyzer



JEOL Spiraltron 17m


(a)
Bradikinin1-7
F
Angiotensin I
Angiotensin II
P14R
ACTH18-39
220 240 260 280 300 320 340 360 380 400 420 440
TOF (μs)
(b)
661.0 661.2 661.4 661.6 661.8
TOF (μs)


Mass resolving power
90000
80000
70000
60000
50000
40000
30000
20000
700
900
1100
1300
1500
1700
1900
2100
2300
2500
m/z
20ns
30ns
40ns
50ns
60ns
70ns

High Resolution MALDI

- Resolving power >20,000 for peptides and small molecules is routine
- Resolving Power approaching 100,000 has been demonstrated with long flight distances
- RMS Mass error 1-5 ppm across the plate over the full mass range with single peak automatic calibration is routine
- MALDI ions often metastable thus longer flight time imply lower sensitivity particularly for larger, more fragile ions
- Sensitivity generally limited by chemical noise, thus higher resolving power may yield higher sensitivity

For Linear Analyzer (size matters more)

Initial position, δx: $R_{s1} = 2[(D_v-D_s)/2d_0y](\delta x/D_e)$

Initial velocity, δv_0: $R_{v1} =(4d_0y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

~~$R_{v2} =2[2d_0y/(D_v-D_s)]^2 (\delta v_0/v_n)^2=0$~~

~~$R_{v3} =2[2d_0y/(D_v-D_s)]^3 (\delta v_0/v_n)^3$~~

Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

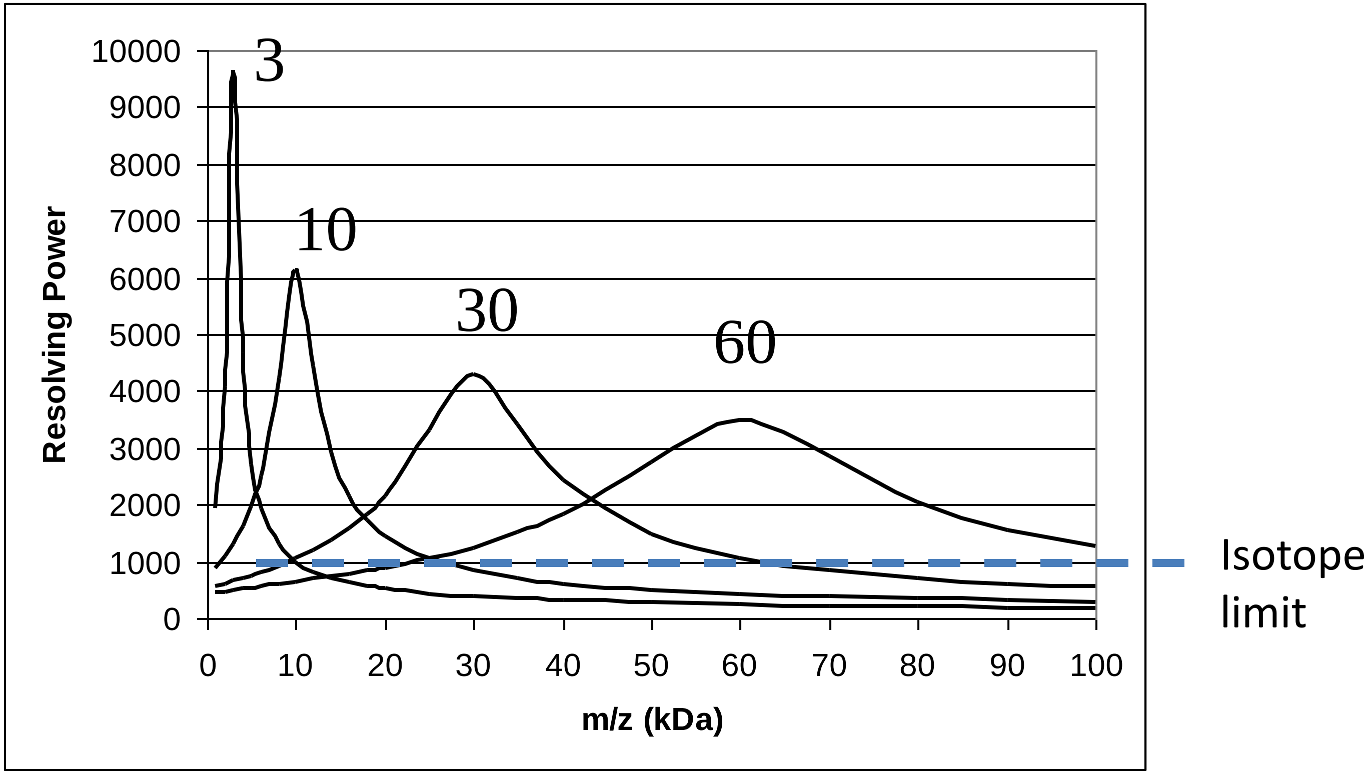
Trajectory error, δL: $R_L = 2\delta L/D_e$

~~Voltage error, δV: $R_V = \delta V/V$~~

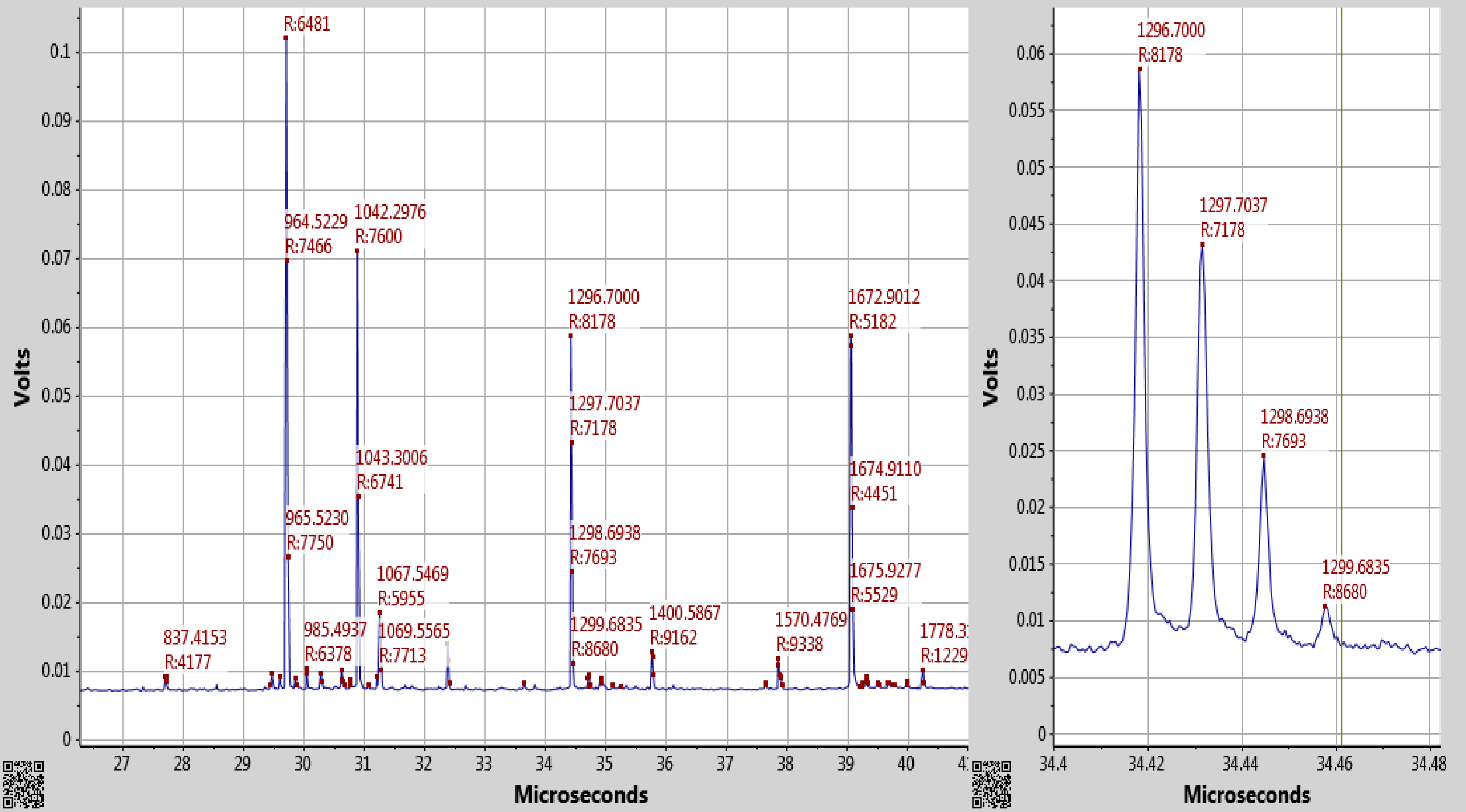
Resolving power:

$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$

Sensitivity, Dynamic Range, and Reproducibility are Key Metrics

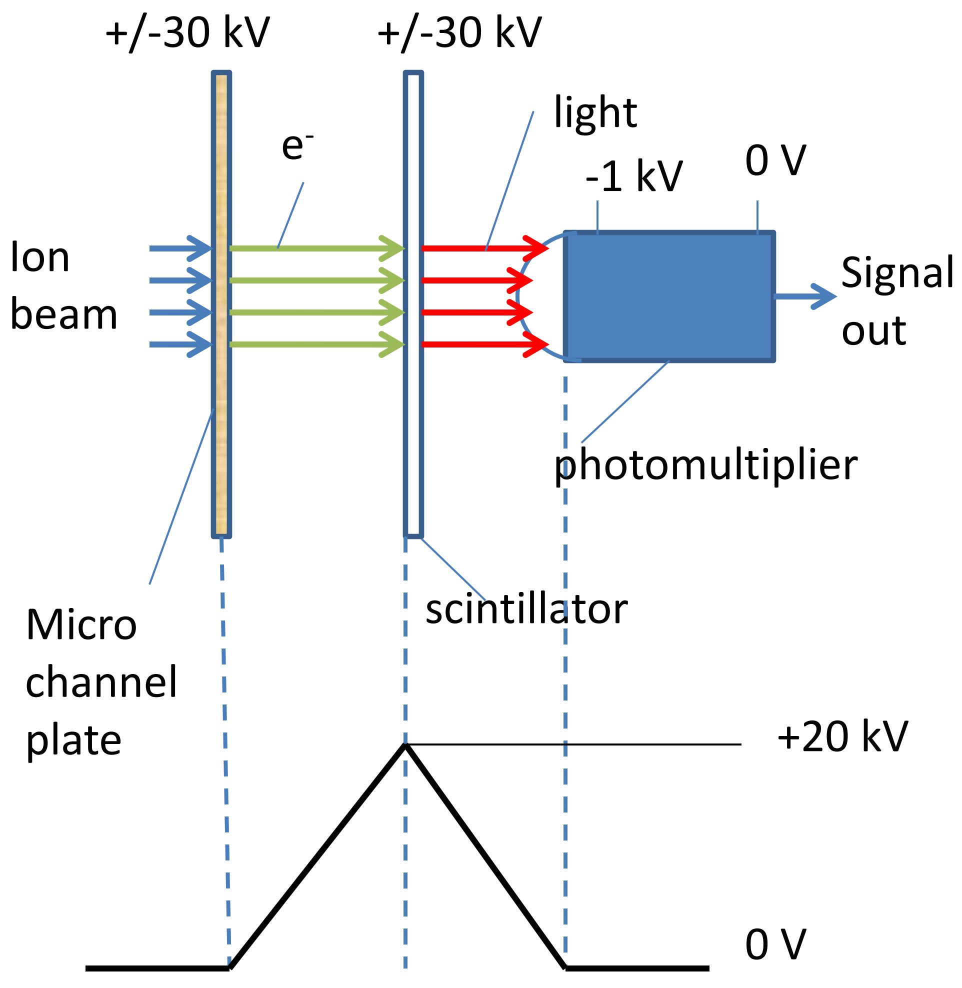


Maximum Resolving Power for Linear Analyzer.



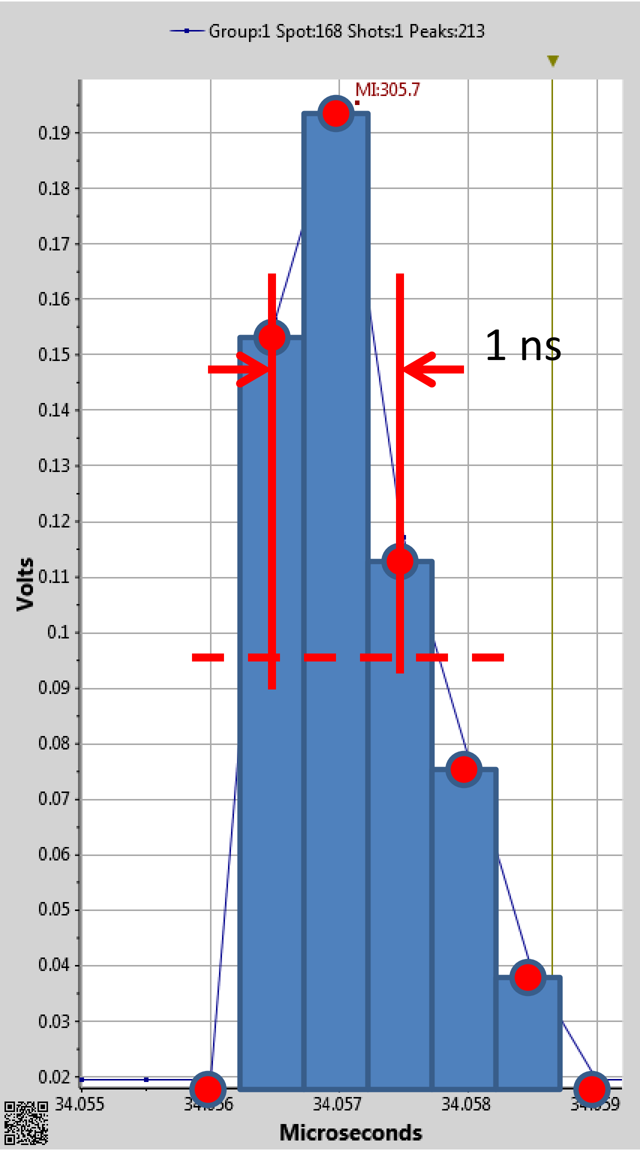
Linear @10 kV -- Higher Resolving Power than the old XL Reflector

Photonis detector



Potential diagram for linear detector

Typical single ion pulse with fast scintillator



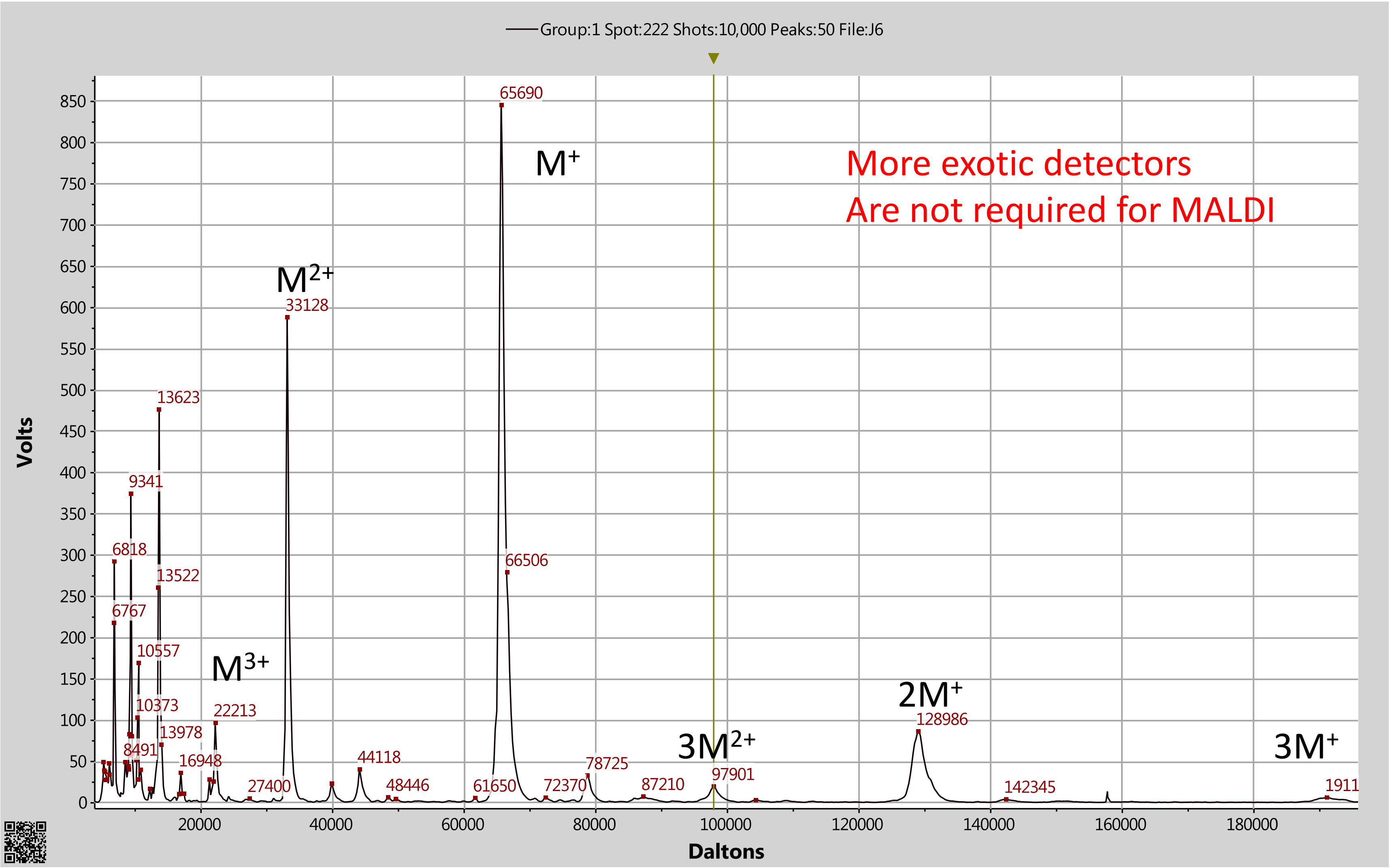
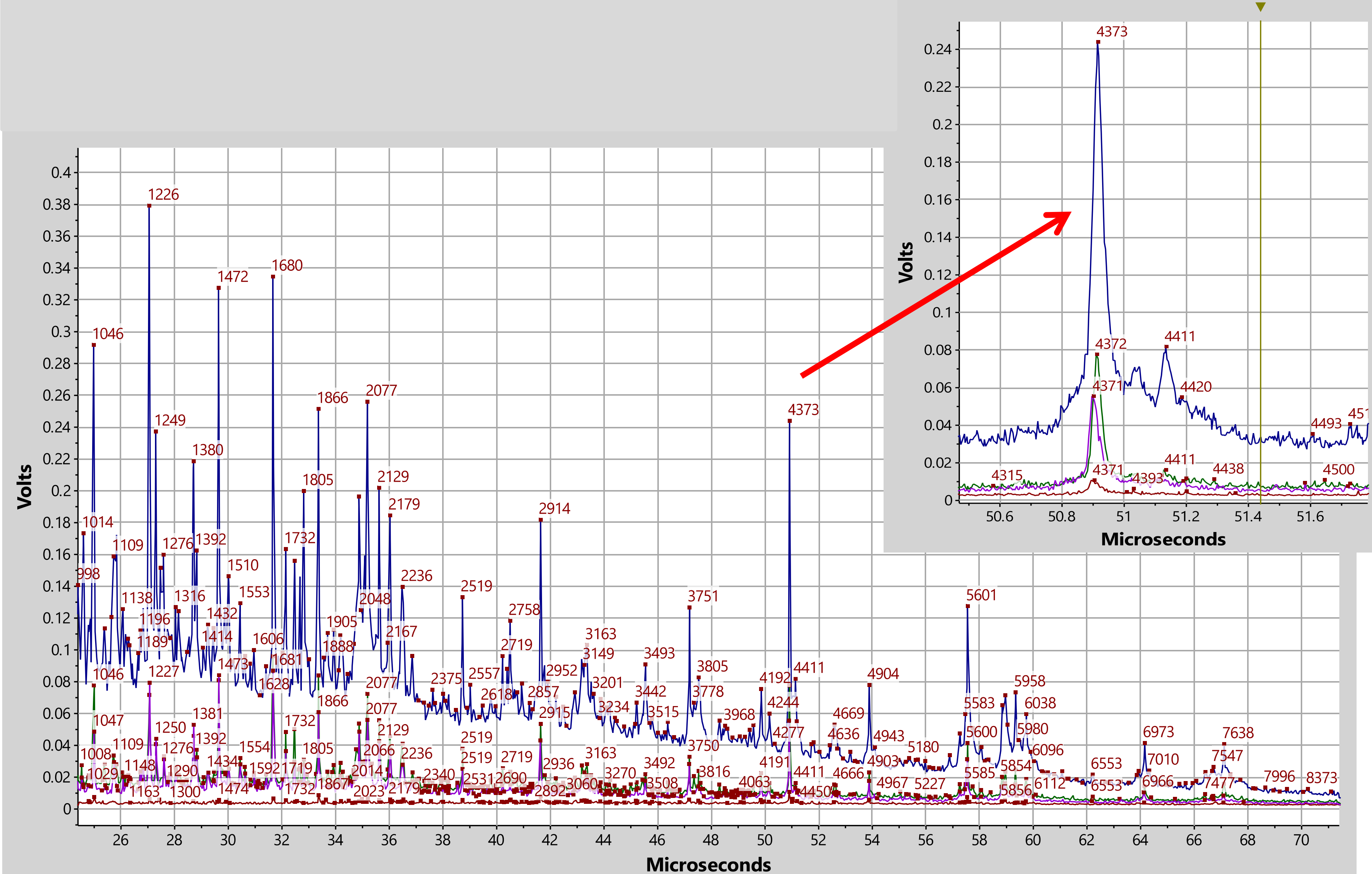


Fig. 9 BSA (1 picomole/μl) in sinnipinic acid matrix 10,000 laser shots in 2 s.

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

Can MALDI be Quantitative????

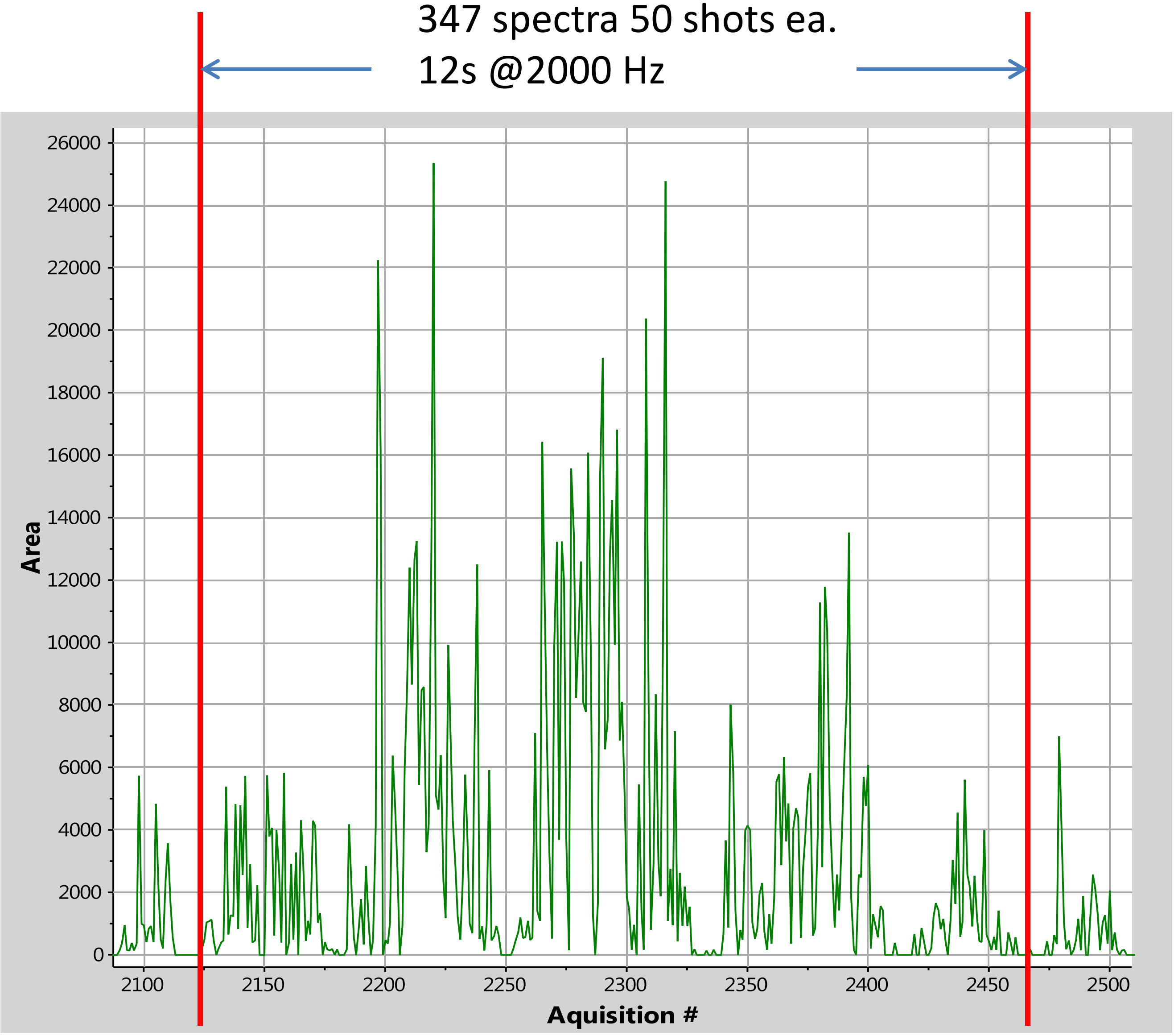
Data Acquisition, One Example

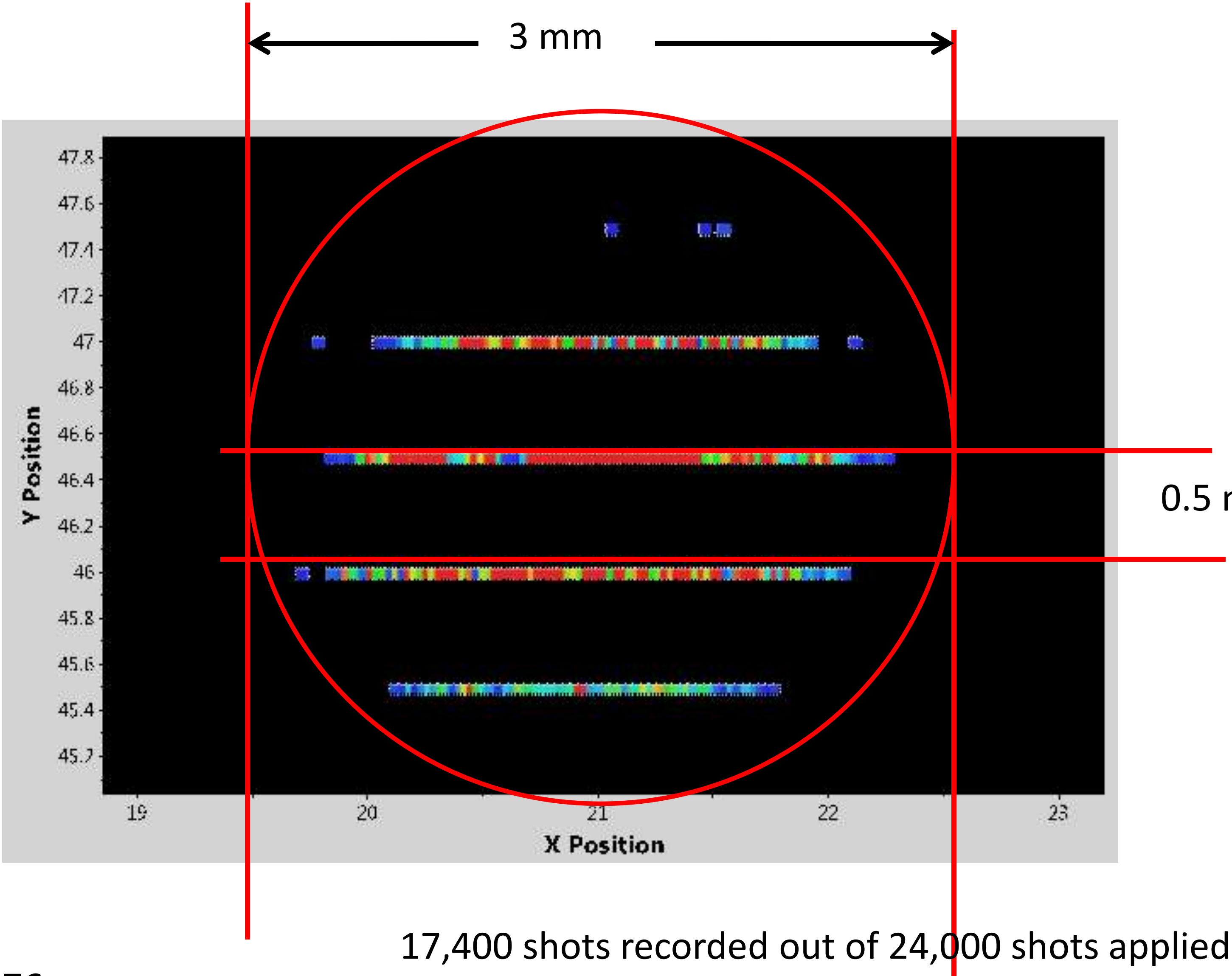
Data is acquired over mass range 1000-20,000 Da using 2 ns bins
2 kHz laser rate
50 shots averaged per spectrum
1 mm/s snake raster for 5 passes at 500 mm over 3 mm dia spot
Total travel 12 mm in 12 s generating 480 spectra with 50 μm resolution
Spectra with no significant peaks are not saved
Only 10%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

Data Processing

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

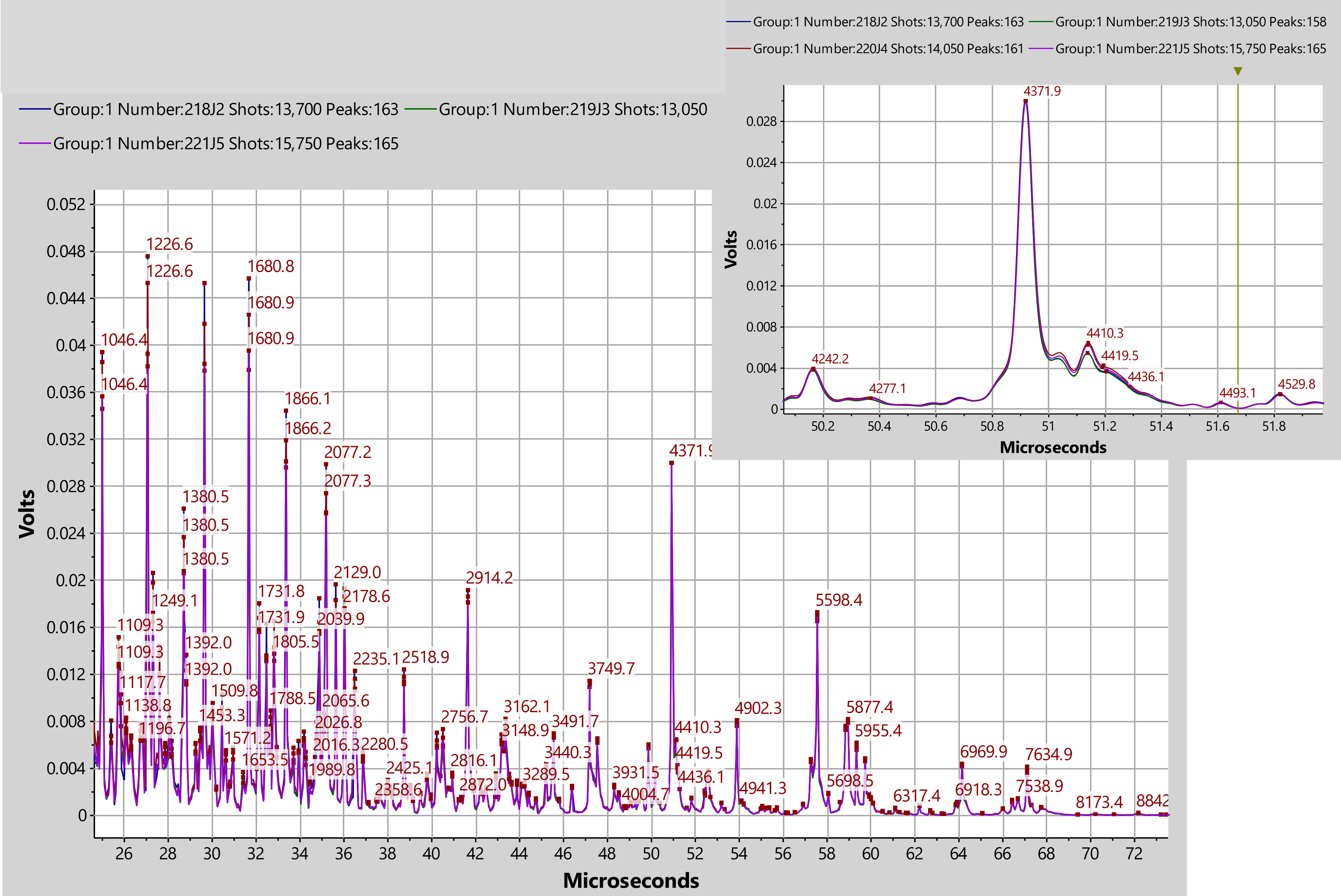
347 spectra 50 shots ea.
12s @2000 Hz





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

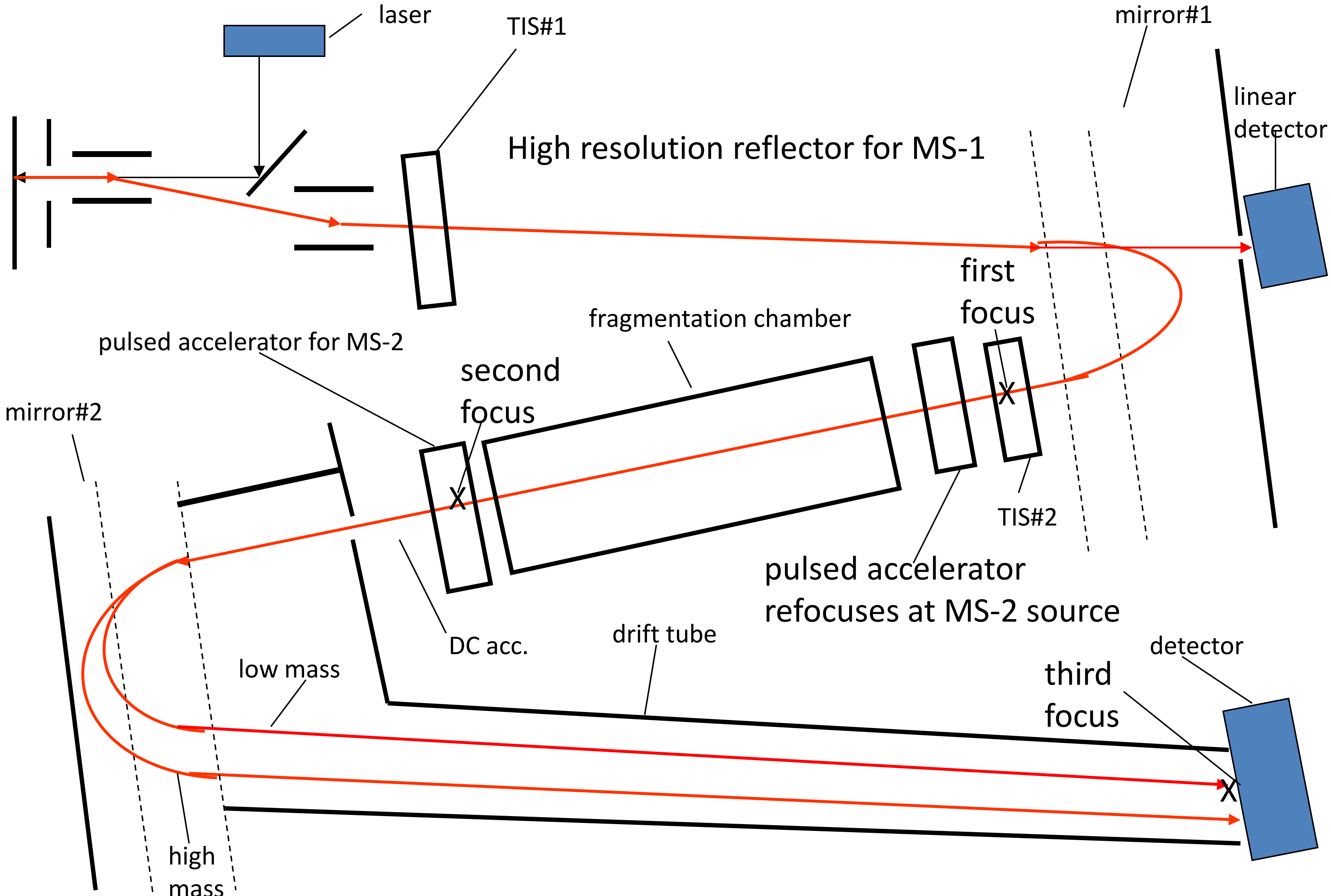
Spot 176



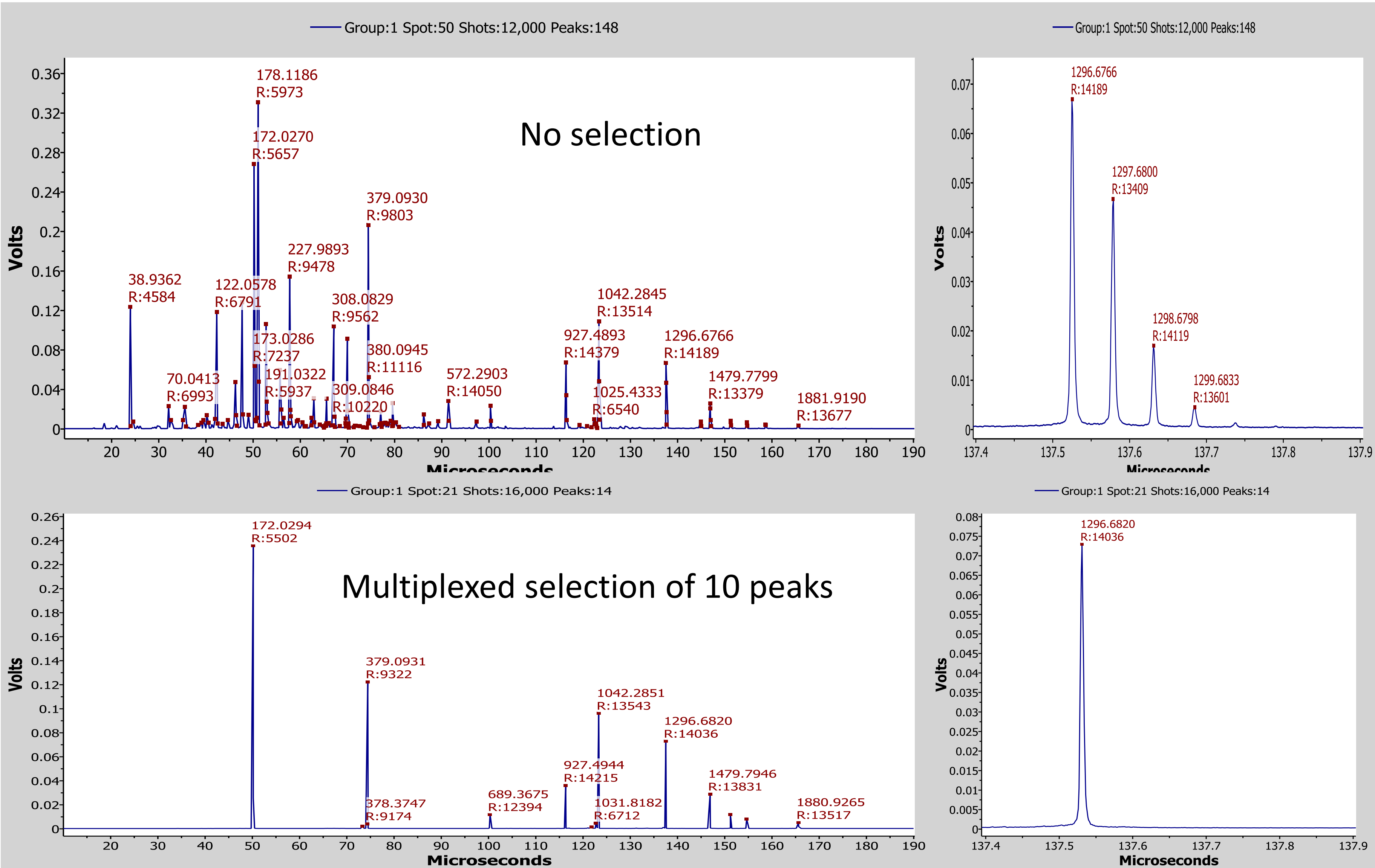
Spectra from 4 different sample spots super imposed

Linear MALDI

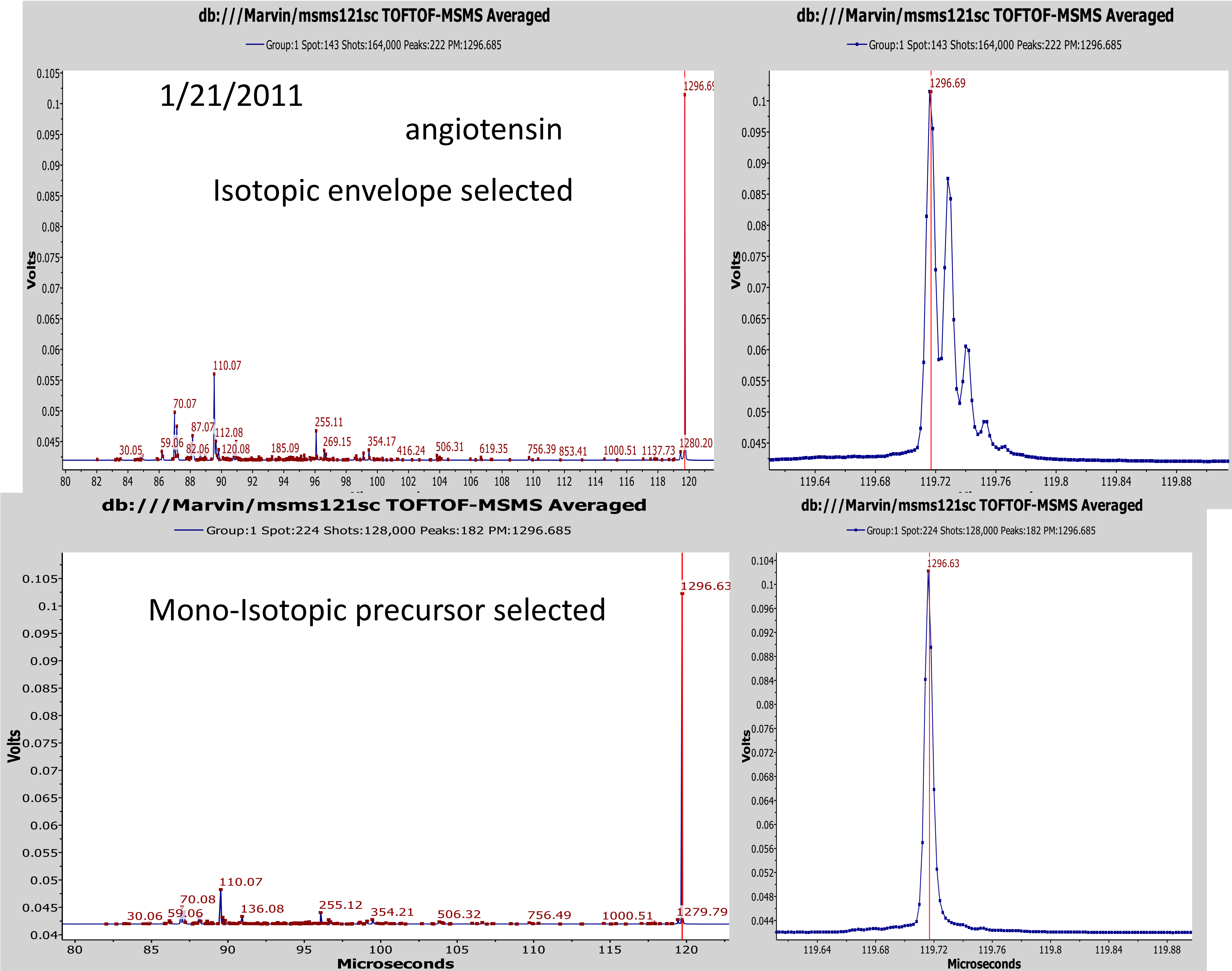
- Resolving power is mass dependent
  - 5000-8000 over limited range at low mass
  - 500-1000 over wide range limited by width of isotope distribution
- Each spot will yield up to 200,000 shots without degrading resolving power or accuracy and giving dynamic range limited only by chemical noise
- Mass error <30 ppm across the plate over the full mass range with single peak automatic calibration
- Dynamic range up to100,000
- High laser rate and data processing makes MALDI quantitative



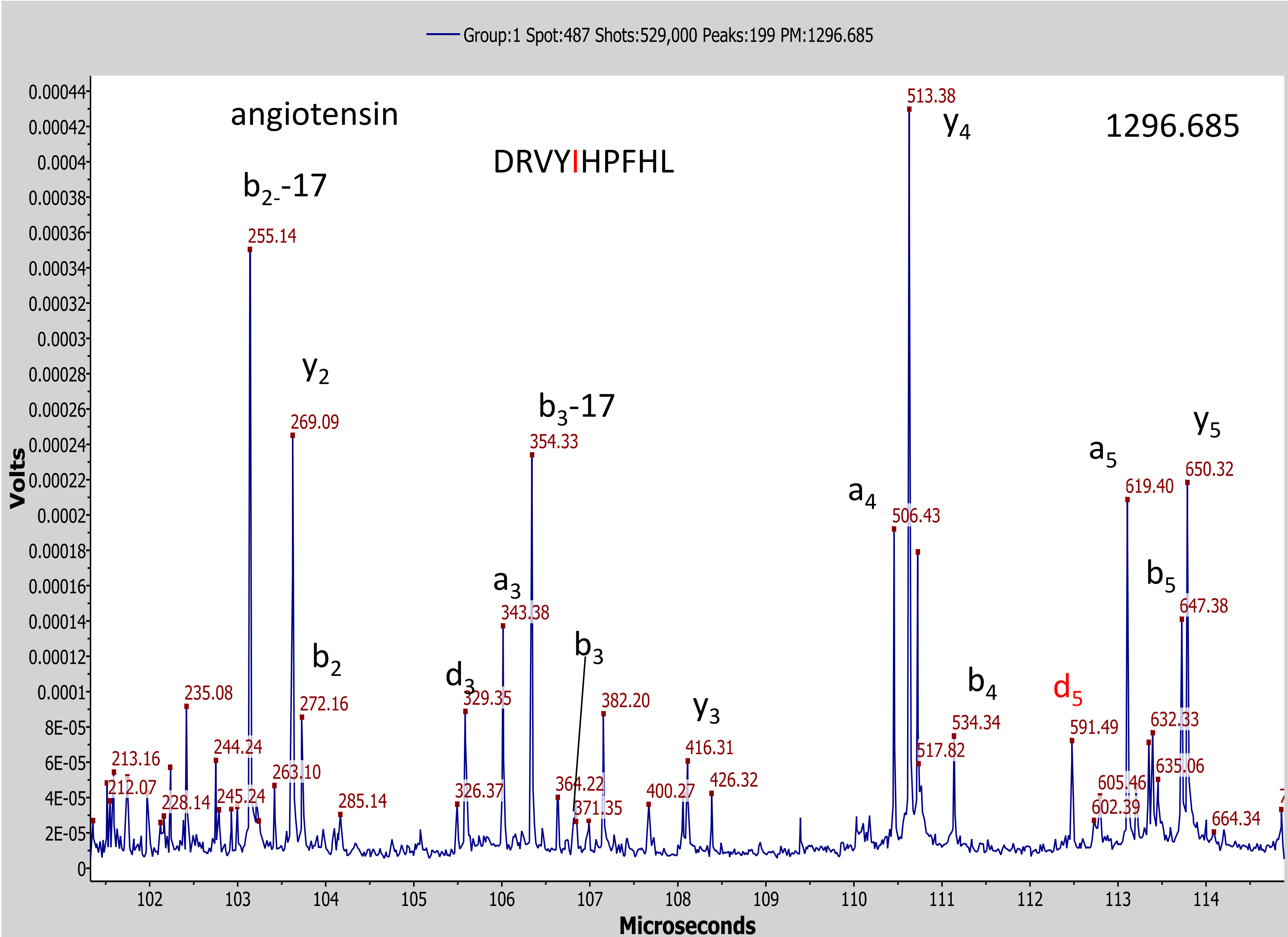
Dual mirror TOF-TOF

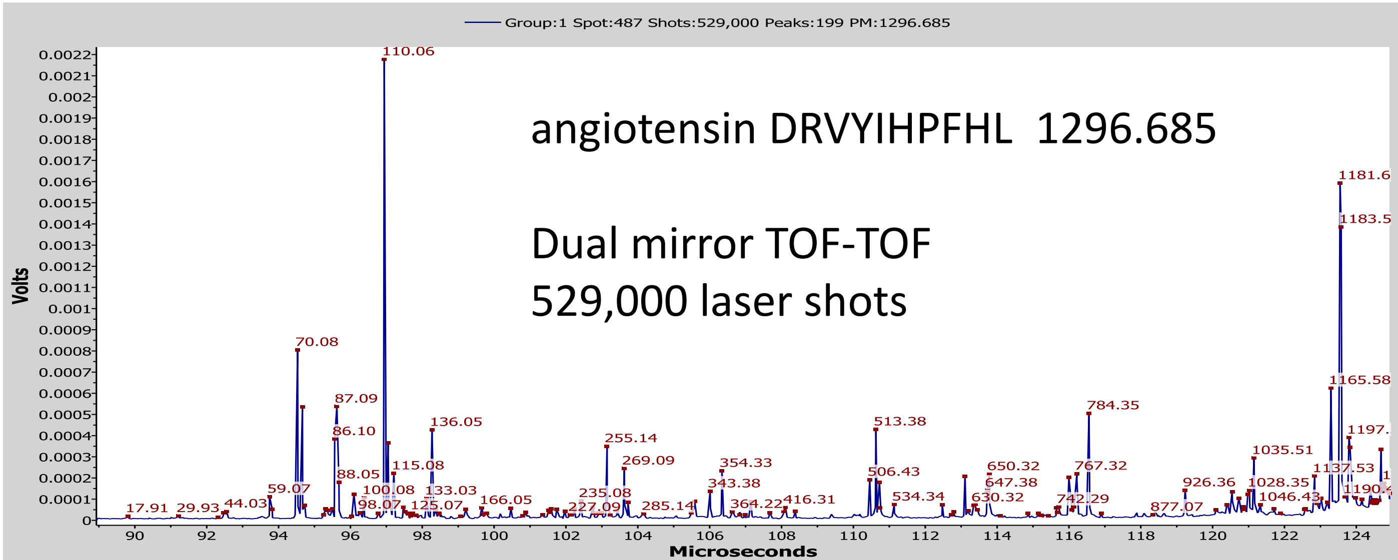


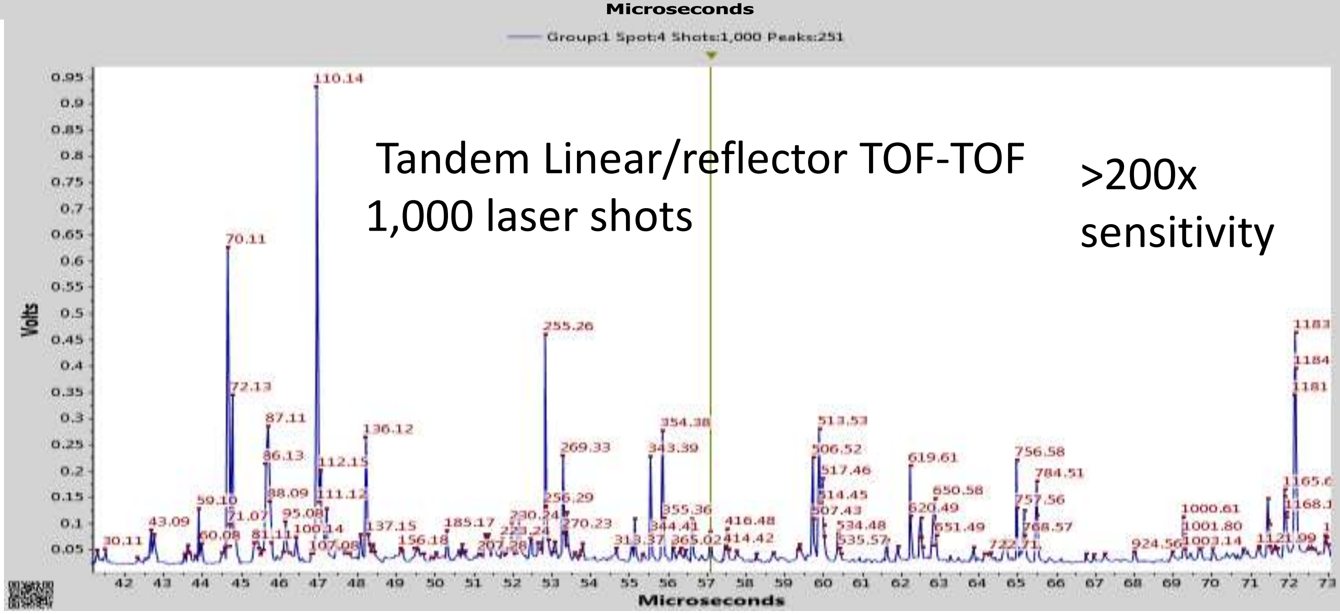
Group:1 Spot:50 Shots:12,000 Peaks:148
No selection
178.1186 R:5973
172.0270 R:5657
379.0930 R:9803
227.9893 R:9478
38.9362 R:4584
122.0578 R:6791
308.0829 R:9562
1042.2845 R:13514
173.0286 R:7237
927.4893 R:14379
1296.6766 R:14189
380.0945 R:11116
70.0413 R:6993
191.0322 R:5937
572.2903 R:14050
1479.7799 R:13379
309.0846 R:10220
1025.4333 R:6540
1881.9190 R:13677
Volts
Microseconds
1296.6766 R:14189
1297.6800 R:13409
1298.6798 R:14119
1299.6833 R:13601
Group:1 Spot:21 Shots:16,000 Peaks:14
Multiplexed selection of 10 peaks
172.0294 R:5502
379.0931 R:9322
1042.2851 R:13543
1296.6820 R:14036
927.4944 R:14215
1479.7946 R:13831
378.3747 R:9174
689.3675 R:12394
1031.8182 R:6712
1880.9265 R:13517
1296.6820 R:14036



db:///Marvin/msms121sc TOFTOF-MSMS Averaged
Group:1 Spot:143 Shots:164,000 Peaks:222 PM:1296.685
1/21/2011
angiotensin
Isotopic envelope selected
Volts
1296.69
110.07
70.07
87.07
112.08
59.06
82.06
120.08
30.05
185.09
255.11
269.15
354.17
416.24
506.31
619.35
756.39
853.41
1000.51
1137.73
1280.20
db:///Marvin/msms121sc TOFTOF-MSMS Averaged
Group:1 Spot:143 Shots:164,000 Peaks:222 PM:1296.685
Volts
1296.69
db:///Marvin/msms121sc TOFTOF-MSMS Averaged
Group:1 Spot:224 Shots:128,000 Peaks:182 PM:1296.685
Mono-Isotopic precursor selected
Volts
1296.63
110.07
70.08
30.06
59.06
136.08
255.12
354.21
506.32
756.49
1000.51
1279.79
Microseconds
db:///Marvin/msms121sc TOFTOF-MSMS Averaged
Group:1 Spot:224 Shots:128,000 Peaks:182 PM:1296.685
Volts
1296.63
Microseconds


Group:1 Spot:487 Shots:529,000 Peaks:199 PM:1296.685
angiotensin
DRVYIHPFHL
1296.685
b_{2-}-17
255.14
y_2
269.09
b_3-17
354.33
y_4
513.38
a_4
506.43
a_5
619.40
y_5
650.32
b_5
647.38
a_3
343.38
b_3
b_2
272.16
d_3
329.35
382.20
y_3
416.31
426.32
b_4
534.34
d_5
591.49
632.33
635.06
605.46
602.39
664.34
235.08
213.16
212.07
228.14
244.24
245.24
263.10
285.14
326.37
364.22
371.35
400.27
517.82
Volts
Microseconds


Group:1 Spot:487 Shots:529,000 Peaks:199 PM:1296.685
angiotensin DRVYIHPFHL 1296.685
Dual mirror TOF-TOF
529,000 laser shots
Volts
Microseconds


Group:1 Spot:4 Shots:1,000 Peaks:251
Tandem Linear/reflector TOF-TOF
1,000 laser shots
>200x
sensitivity
Volts
Microseconds

Status of TOF-TOF

- Trade-off between high resolution precursor selection and high sensitivity for fragments
- 5 kHz laser makes LC-MALDI-MS-MS practical
- Multiplexing up to 10 precursors/laser shot practical for MRM measurements
- Both low energy fragmentation (psd) and high energy(cid) are practical
- Still not widely accepted relative to ESI with traps

Applications

- 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
- Protein array reader