New MALDI-TOF MS with very high resolving power and mass accuracy

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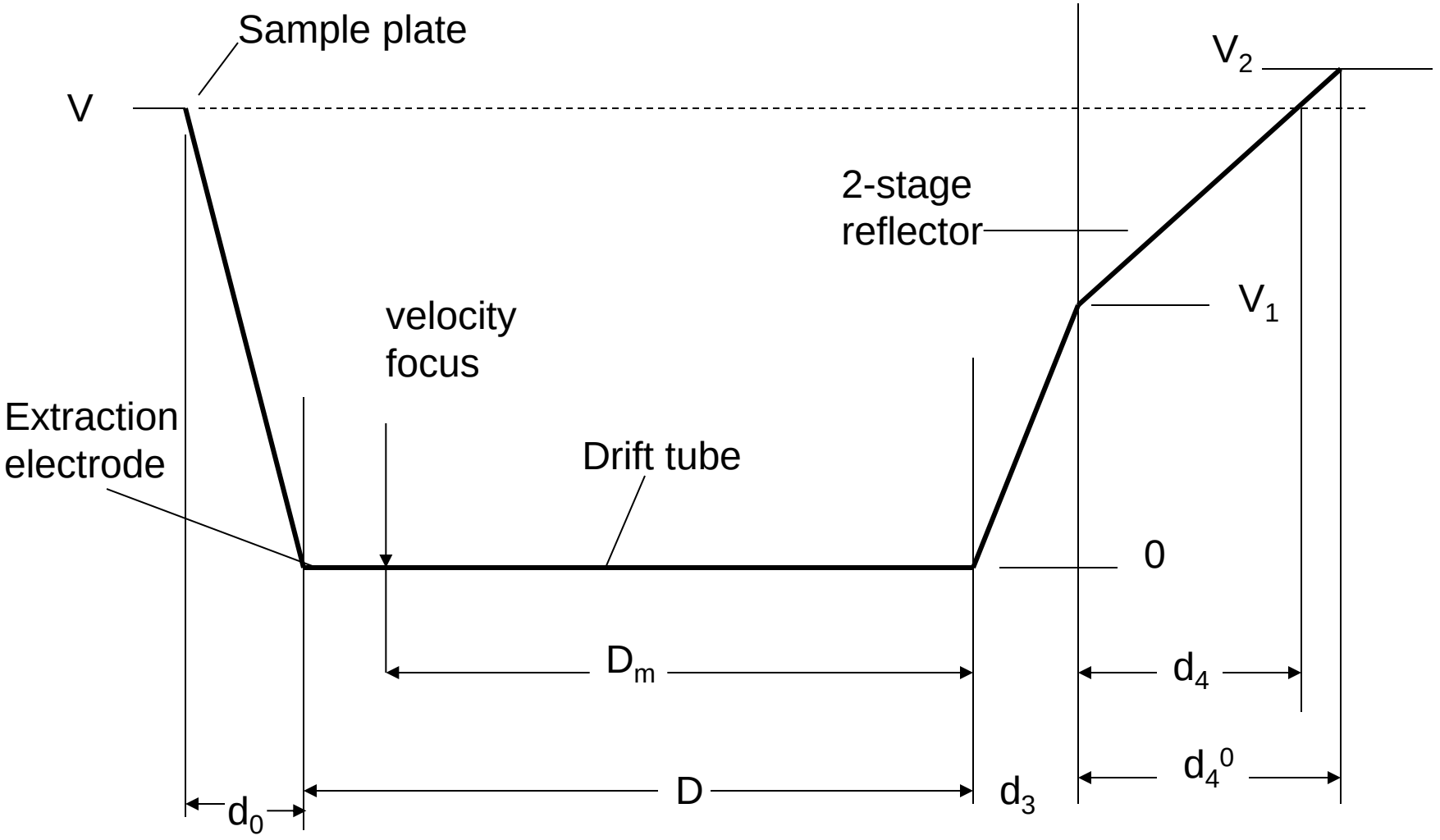
Objectives

- Determine optimum design parameters for a high-performance bench-top MALDI-TOF
  - Resolving power and mass accuracy
  - Sensitivity and dynamic range
  - High throughput
  - Efficient sample utilization
  - Simple and reliable
  - Low cost
- Build prototype instrument and compare experimental results with theoretical predictions

Common Features of Virgin MALDI TOF

- Simplified sample loading with super microplate format (102x108 mm)
- Benchtop cabinet
  - 1.75m high x 0.5 m wide x 0.7 m deep
  - 100 kg max weight
- 5 kHz laser, up to 100 spectra/sec
- Fully automated, no operator expertise required
  - No joystick, no camera
- 24/7 operation with minimal preventative maintenance



Potential diagram for high-resolution TOF with single-field source and two-field reflector. The field-free distance D is the distance from the source to the mirror plus the distance from the mirror to the detector (not shown).

Instrument Parameters for Reflecting Analyzer (single-field source and two-field mirror)

D_e=3200, d_0=6, D=2264, d_3=114.2, d_4^0=127.7 all in mm
V=8.56, V_1=6.31, V_2=9.23 all in kV
δt=1.5 nsec (0.5 nsec bins, 1 nsec single ion pulse width)
Focus mass m^*= 3 kDa
$D_v=6d_0$ = 36 mm, v_n (for m^*)=0.0235 mm/nsec
Time lag $\Delta t=d_0/v_n$ = 250 nsec
$K=2d_0/(D_v-D_s)$=0.5

Initial Conditions for MALDI (typical)

δv_0=400 m/sec, δx=0.01 mm

Trajectory error and voltage error assumed to be small

References

- M. L. Vestal and P. Juhasz, “Resolution and Mass Accuracy in Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectrometry”, J. Am. Soc. Mass Spectrom. **9**, 892-911 (1998).
- M. L. Vestal and K. Hayden, “High-performance MALDI-TOF mass spectrometry for proteomics”, Int. J. Mass Spectrometry **268**, 83-92 (2007).
- M. L. Vestal and K. Hayden, “High-performance MALDI-TOF Mass Spectrometry”, paper TOFam08:15 presented at 55th ASMS Conference on Mass Spectrometry and Allied Topics, Indianapolis, June 3-7, 2007.
- S. J. Hattan and M. L. Vestal, “Novel 3-D sample plate using monolithic capture media in collimated-hole structures for interfacing high capacity separations with MALDI-TOF”, poster TBP-060 presented at 56th ASMS Conference on Mass Spectrometry and Allied Topics, Indianapolis, June 3, 2008.

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

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

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

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

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

$R_v = R_{v1} + R_{v3}$

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

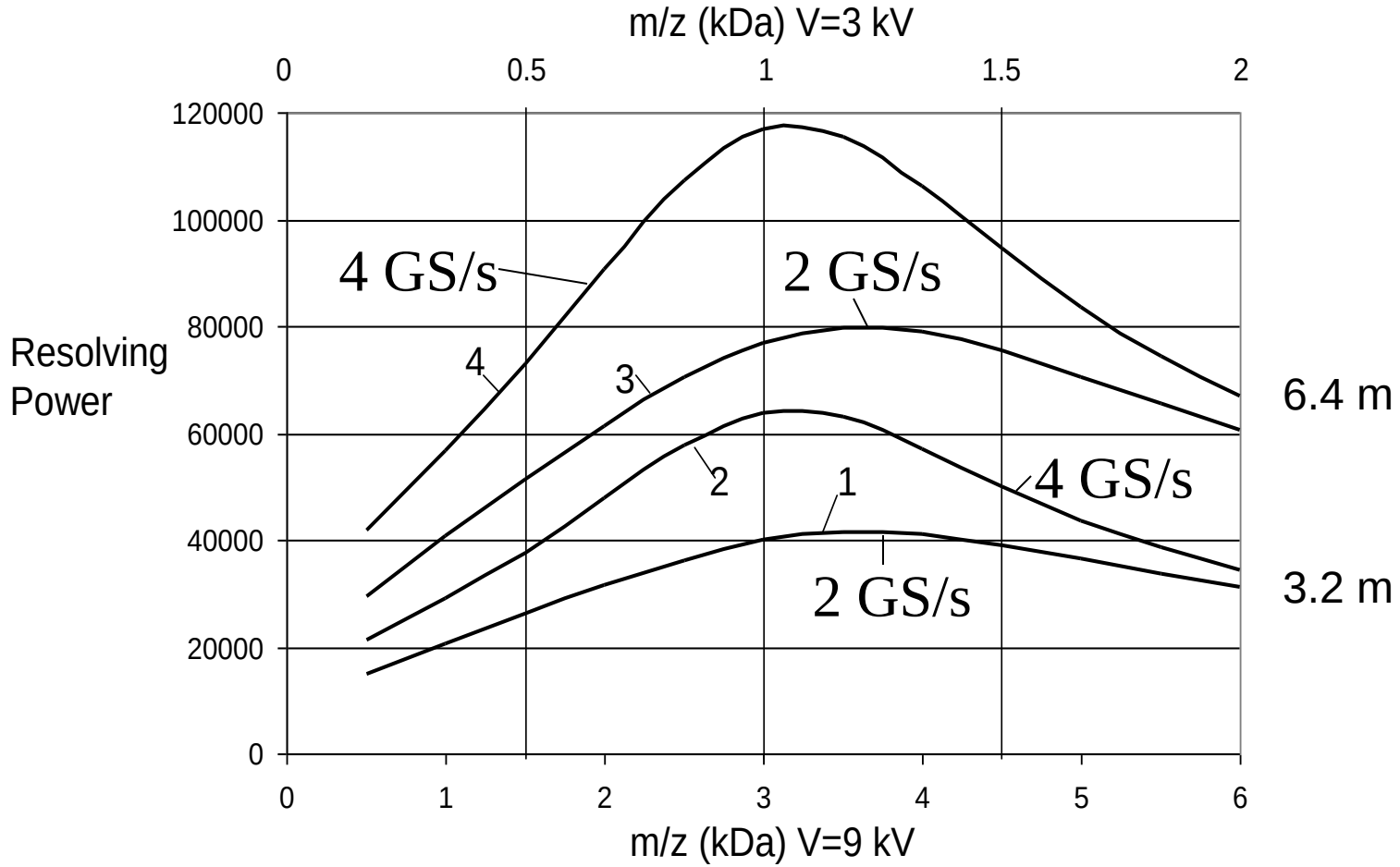
Trajectory error, δL: $R_L = 2\delta L/D_e$

Voltage error, δV: $R_V = \delta V/V$

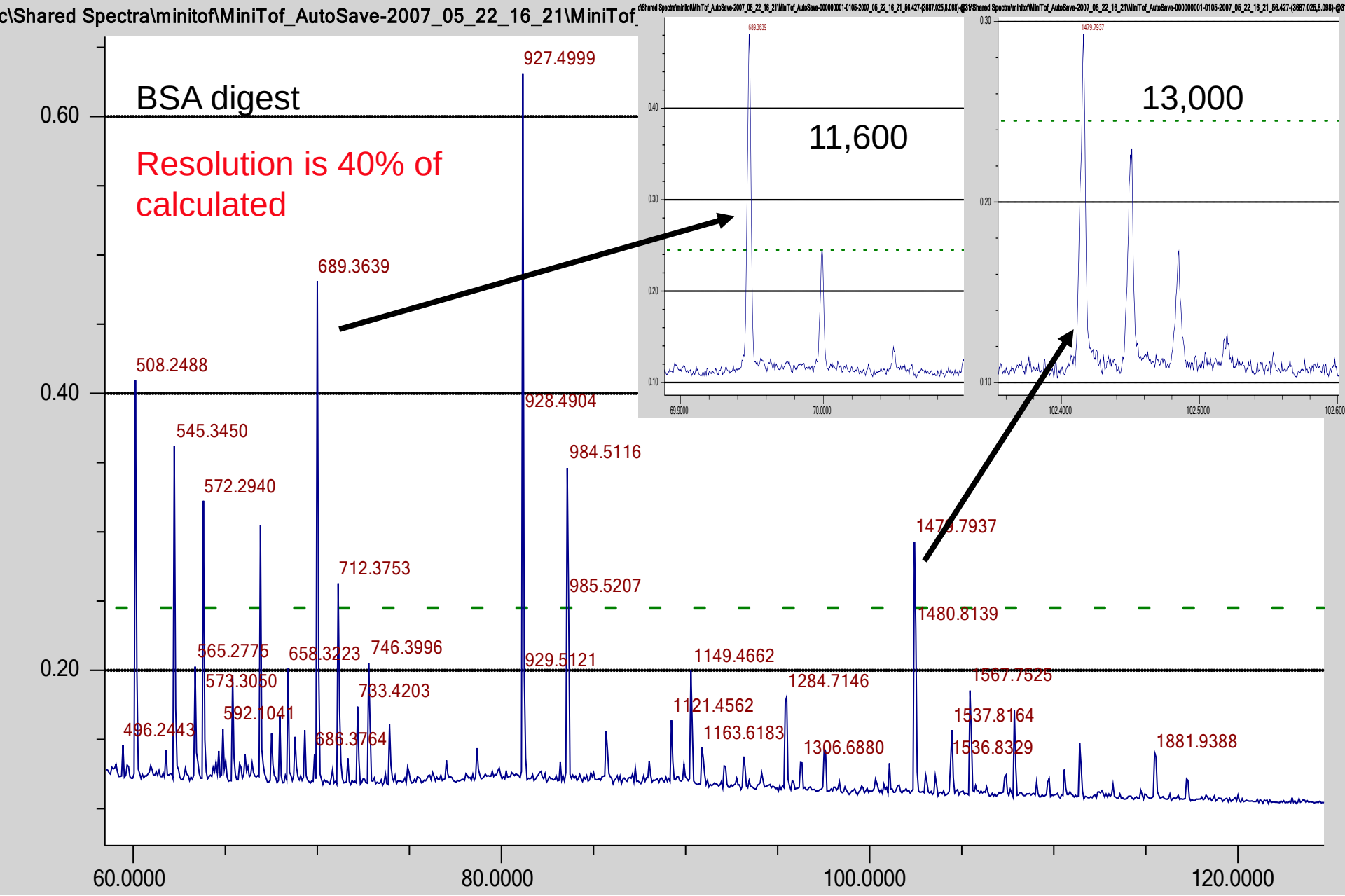
Neglected in initial calculation

Resolving power:

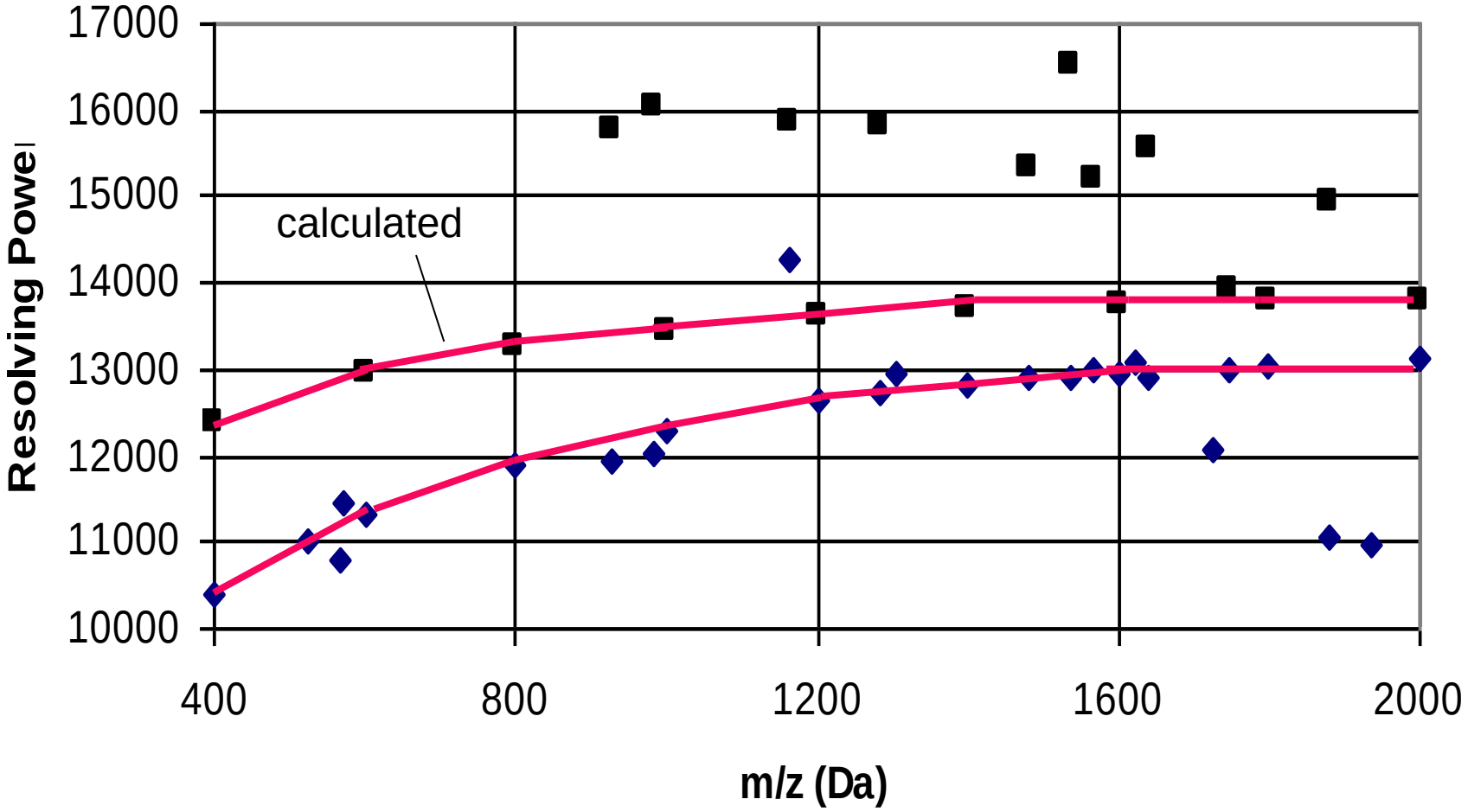
$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$



Calculated resolving power as function of m/z, curve 1 corresponds to experimental instrument



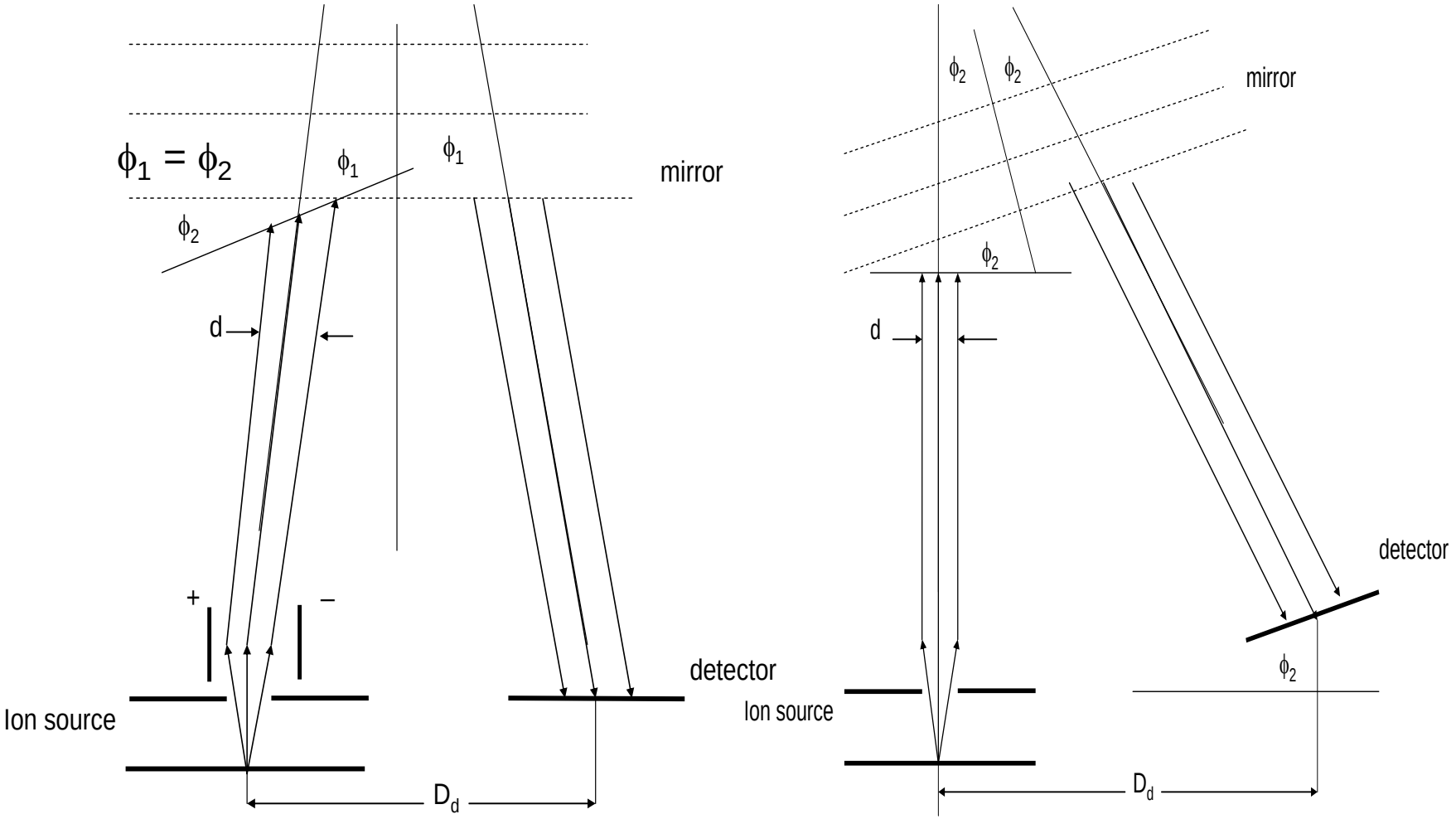
Spectrum from original version



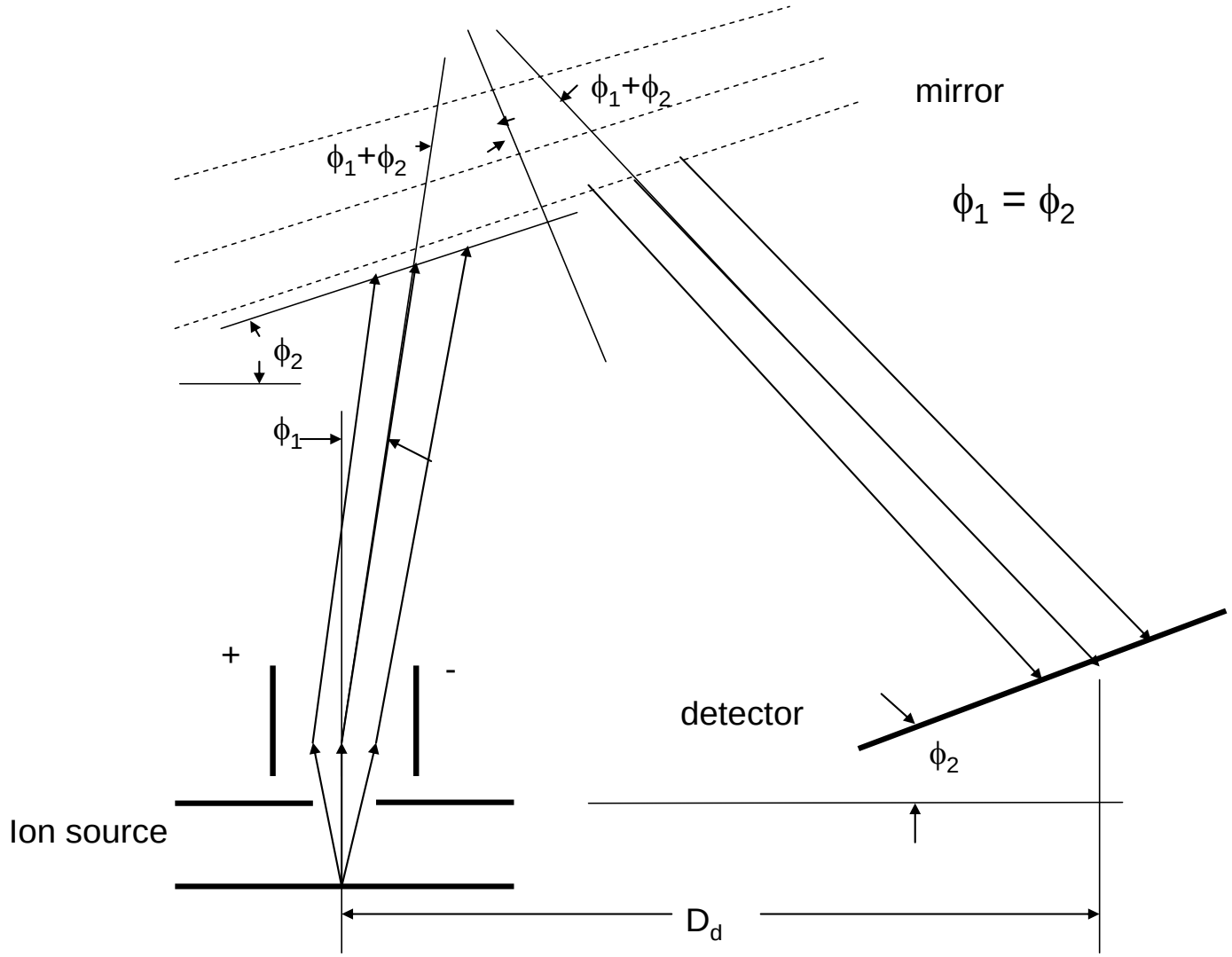
Comparison of calculated resolving power with experimental results
With δm/m=70 ppm due to mass independent errors.
Measured ♦ 5 μchannel plate ■ETP MagnetTOF™ (DM167)
Original version before trajectory error correction

Changes to correct mass independent errors

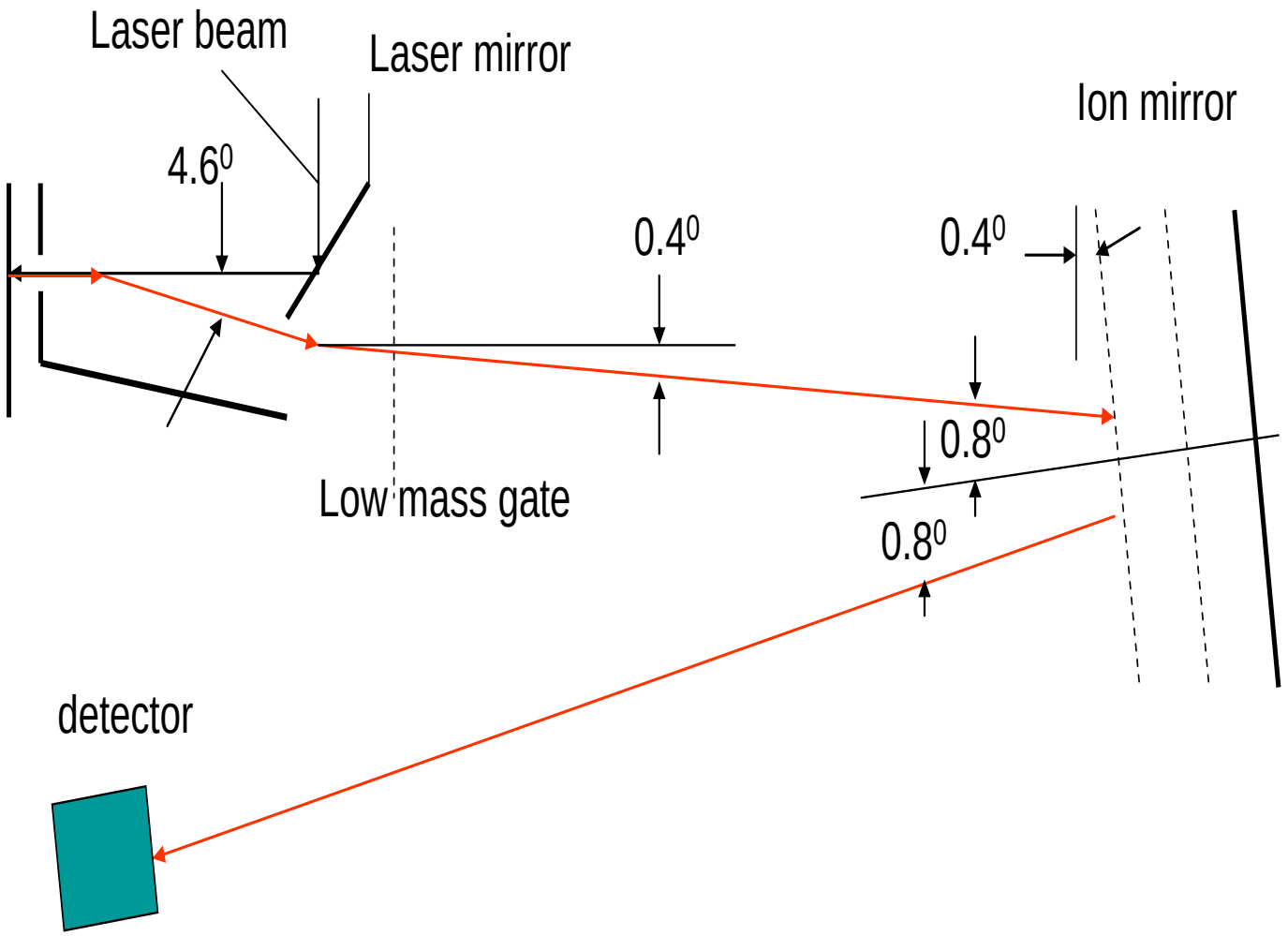
- Add voltage regulator at input to mirror HV supplies (removes low frequency noise)
- Correct trajectory error due to ion deflector
- Use faster detector (0.5 ns)
  - ETP DM167 in place of 5 μm dual channel plate
- Use faster digitizer
  - 0.5 ns bins>0.25 ns bins



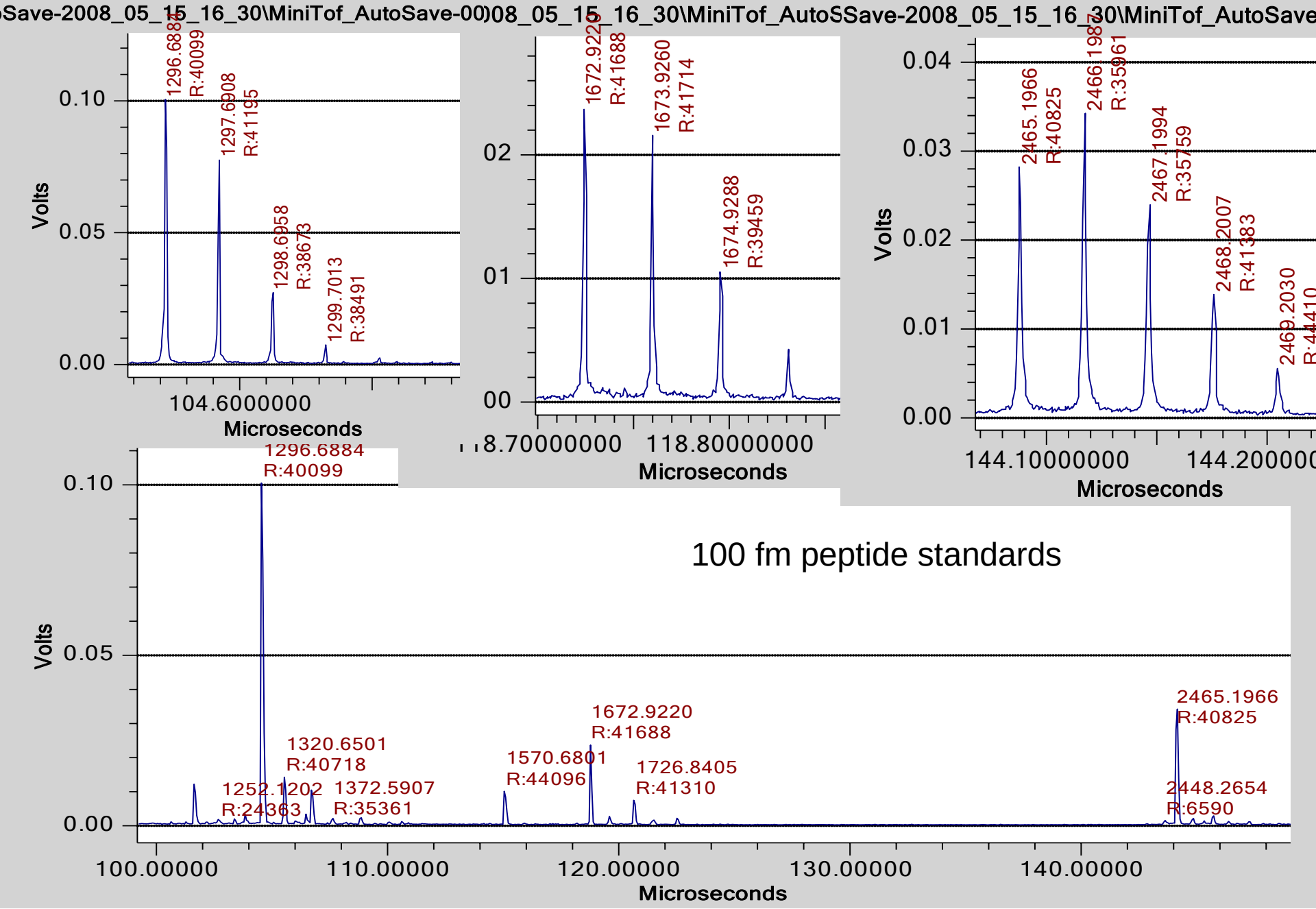
Ray diagrams indicating source of trajectory error



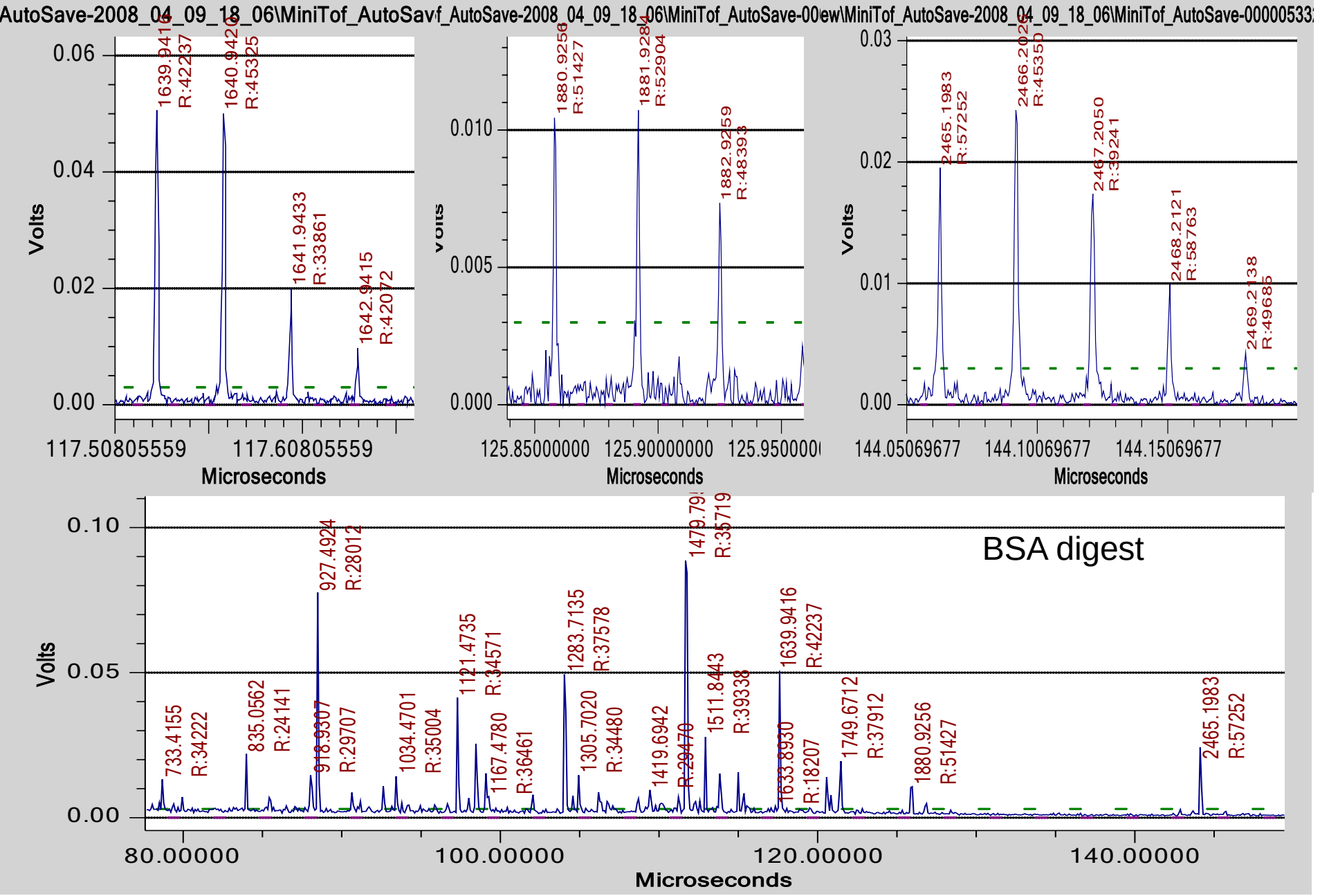
Ray diagram showing general correction to trajectory error due to deflection



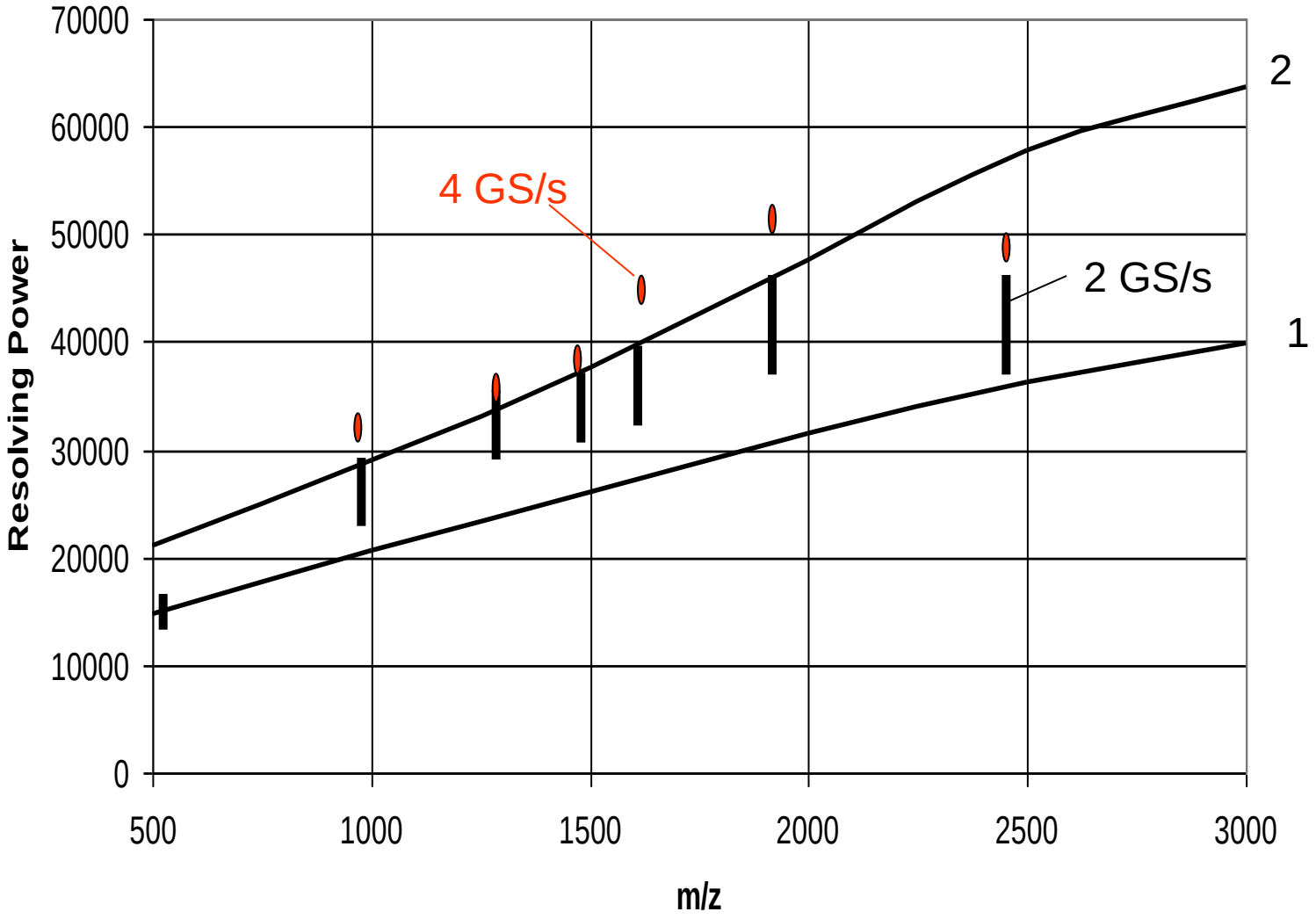
Ray diagram of final version employed in the corrected version of the experimental analyzer



Example of spectrum obtained with corrected analyzer

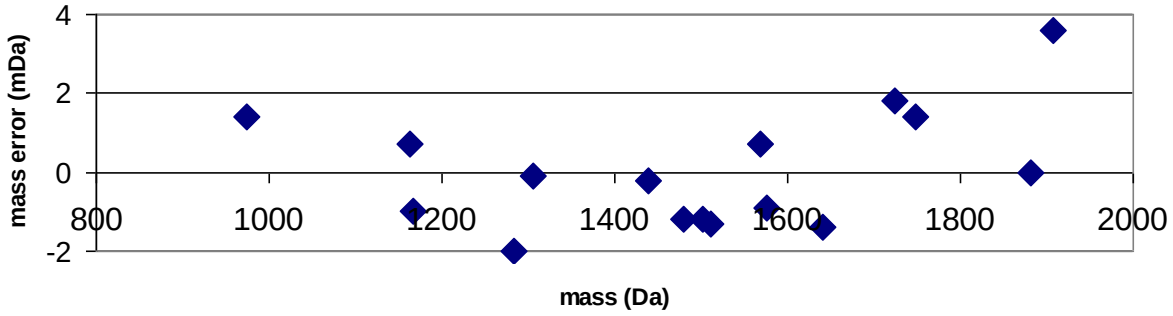


Example of spectrum obtained with corrected analyzer



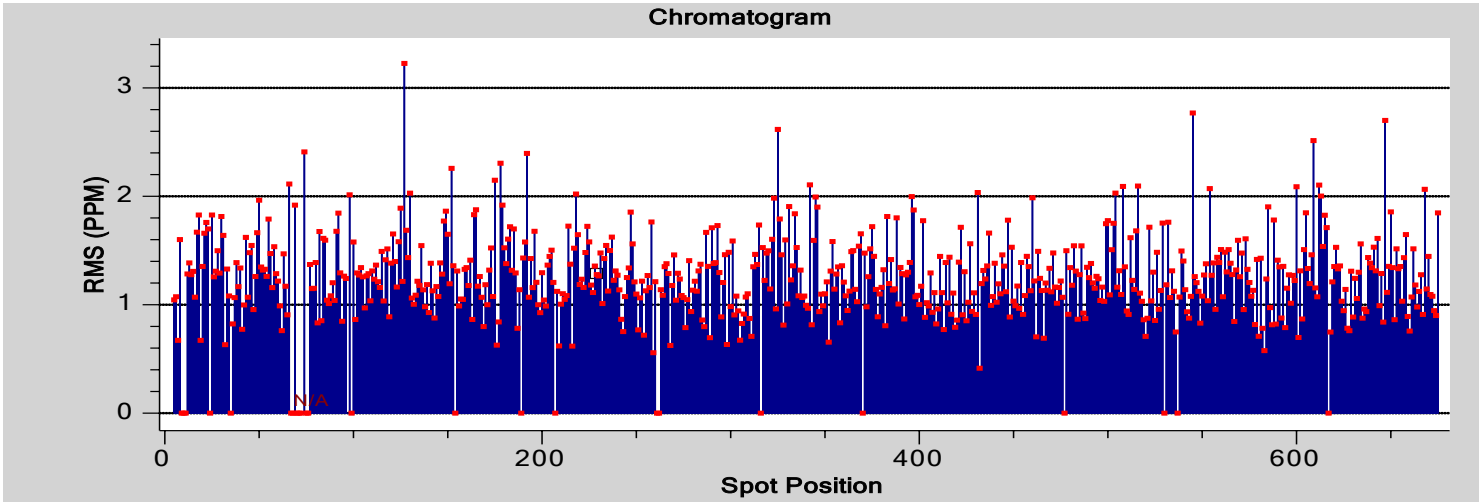
Comparison of experimental results with theoretical calculations. Bars are measured resolving power with standard deviation of 5 replicates. Curve 1 corresponds to digitizer with 0.5 ns bins and curve 2 to 0.25 ns bins.

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 (D_0 and D_1) with D_3 and D_4 fixed.

Serial Dilution Experiment

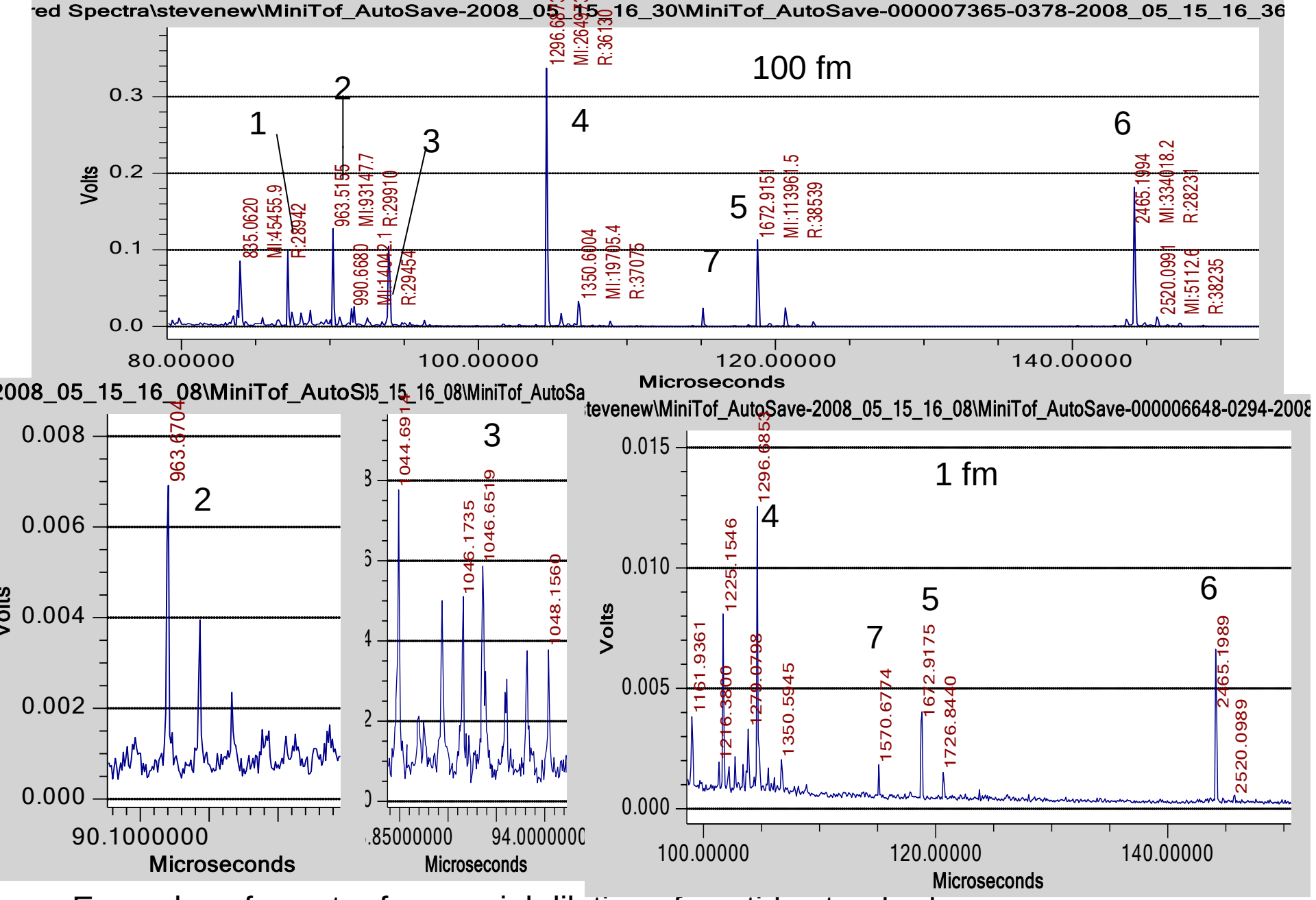
Peptide Standards

	MH^+	No.
Angiotensin Fragment 1-7	899.47	1
des-Pro2 Bradykinin	963.51	2
Angiotensin II Acetate	1046.54	3
Angiotensin I	1296.69	4
Neurotensin	1672.92	5
ACTH (18-39)	2465.20	6
Glu-1 Fibrinopeptide B	1570.68	7(<10% pure)

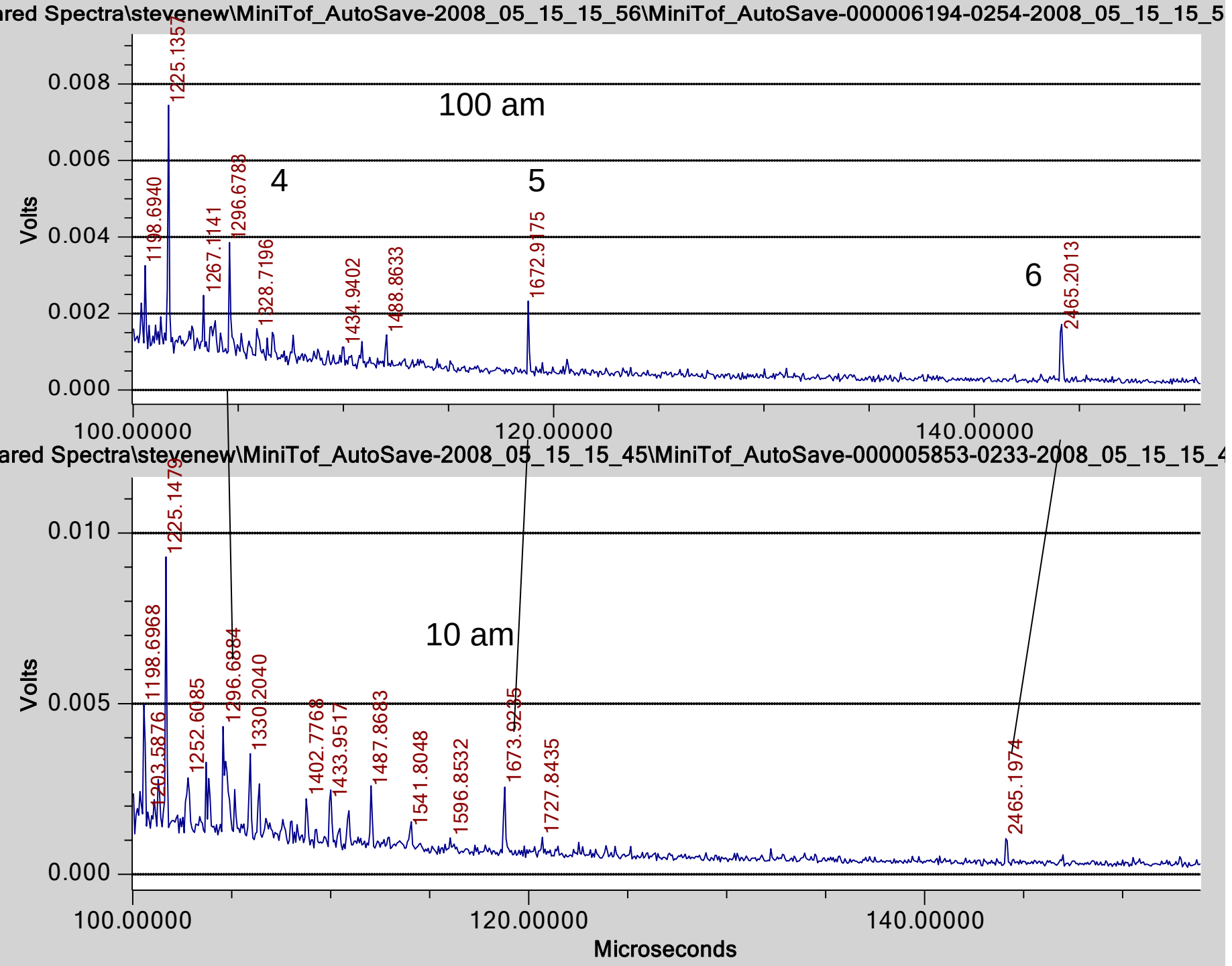
Equimolar mixture diluted in 4-hydroxy-α-cyanocinnamic acid from 1 picomole/μL to 1 attomole/μL

Automated acquisition and calibration averaging 2000 laser shots/spectrum at 1 kHz

No data processing



Examples of spectra from serial dilution of peptide standards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6
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1198.6940
1267.1141
1296.6788
1328.7196
1434.9402
1488.8633
1672.9175
2465.2013
Volts
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0.000
100.00000
120.00000
140.00000
ared Spectra\stevenew\MiniTof_AutoSave-2008_05_15_15_45\MiniTof_AutoSave-000005853-0233-2008_05_15_15_4
10 am
1225.1479
1198.6968
1203.5876
1252.6085
1296.6884
1330.2040
1402.7768
1433.9517
1487.8683
1541.8048
1596.8532
1673.9235
1727.8435
2465.1974
Volts
0.010
0.005
0.000
100.00000
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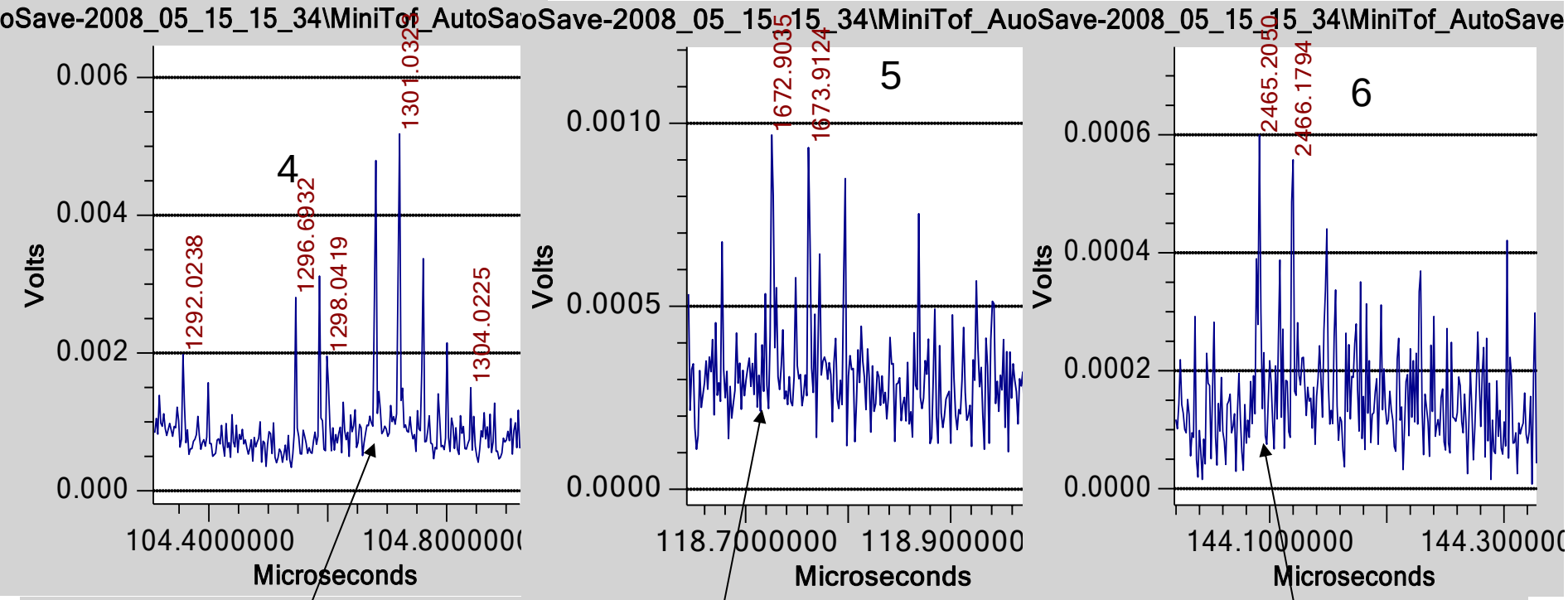
140.00000
Microseconds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oSave-2008_05_15_15_34\MiniTof_AuoSave-2008_05_15_15_34\MiniTof_AutoSave
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1296.6932
1298.0419
1301.0323
1304.0225
1672.9035
1673.9124
2465.2050
2466.1794
0.006
0.004
0.002
0.000
0.0010
0.0005
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0.0002
104.4000000
104.8000000
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118.9000000
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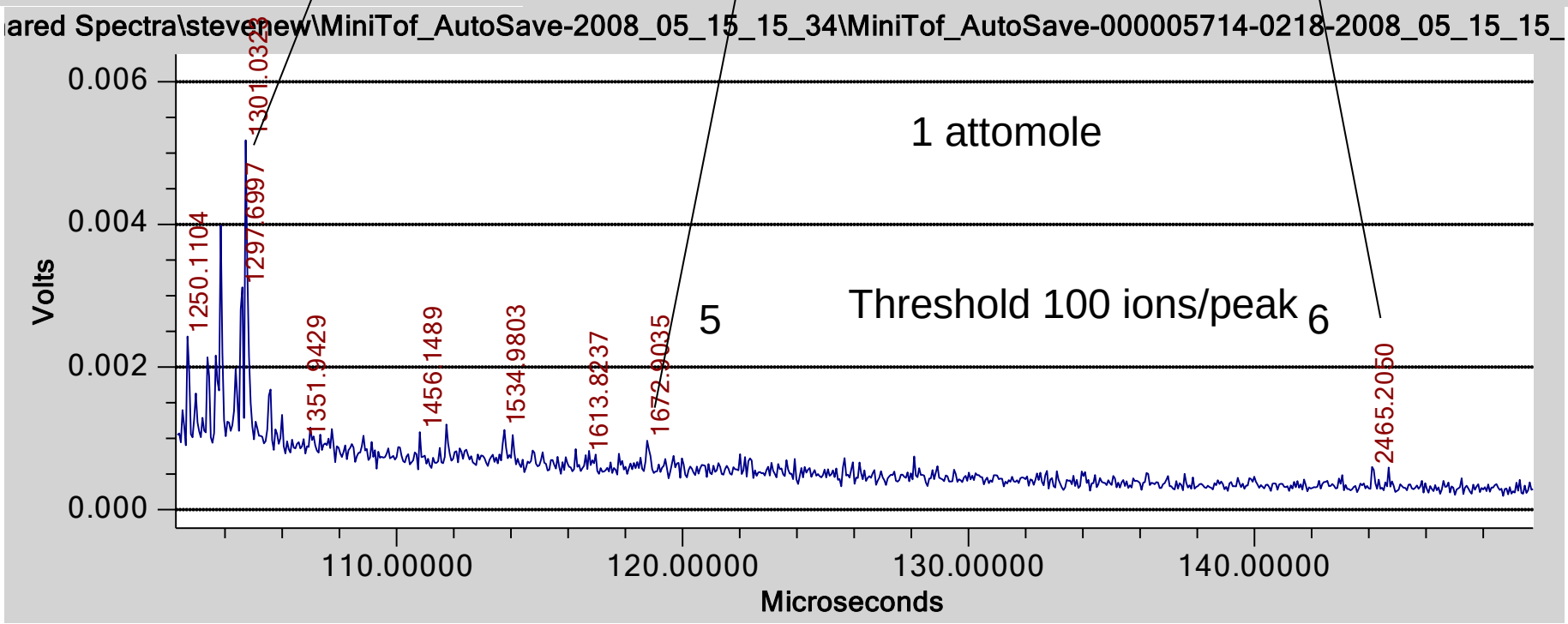
ared Spectra\stevenew\MiniTof_AutoSave-2008_05_15_15_34\MiniTof_AutoSave-000005714-0218-2008_05_15_15_
1 attomole
Threshold 100 ions/peak
5
6
Volts
Microseconds
1250.1104
1297.6997
1301.0323
1351.9429
1456.1489
1534.9803
1613.8237
1672.9035
2465.2050
0.006
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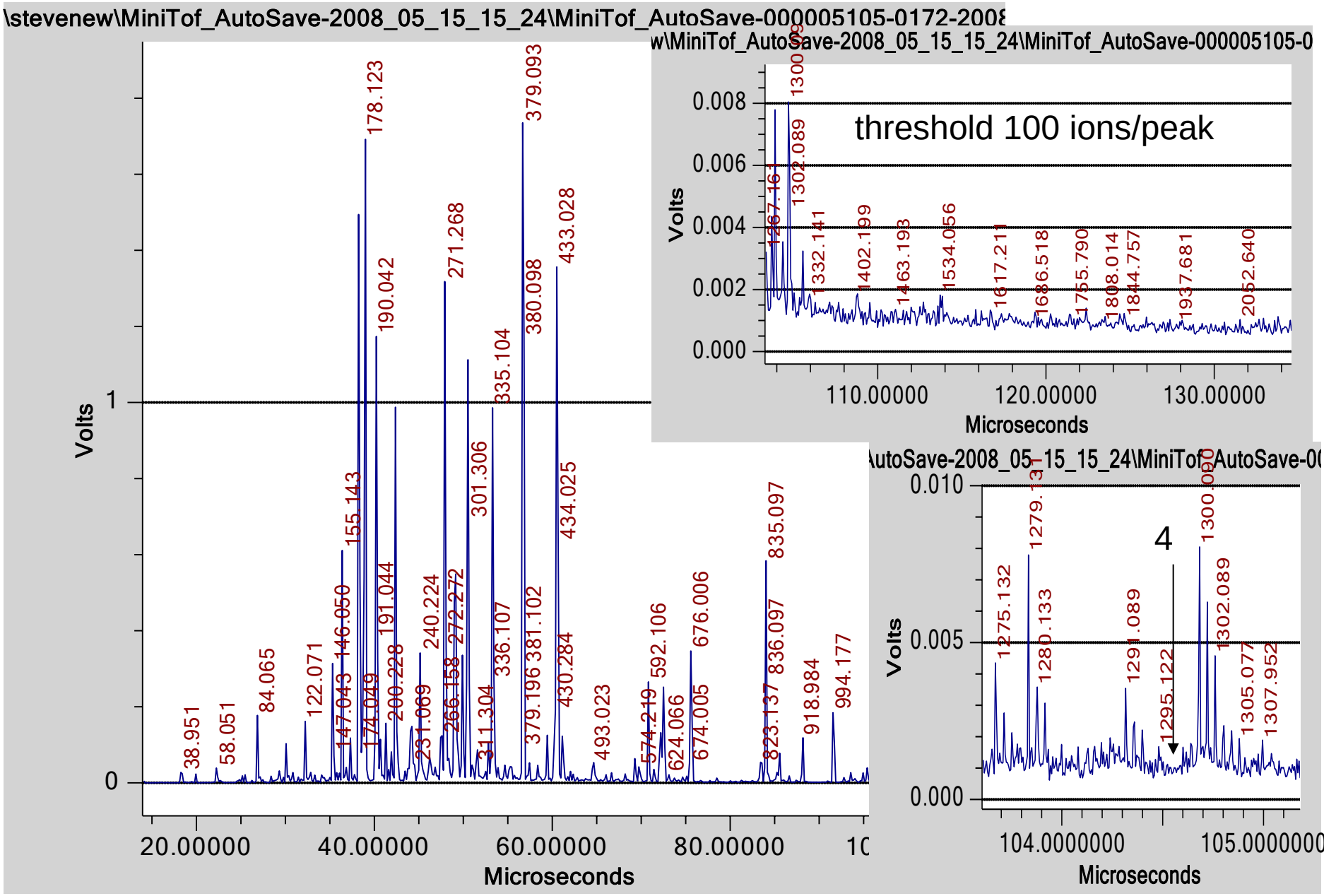
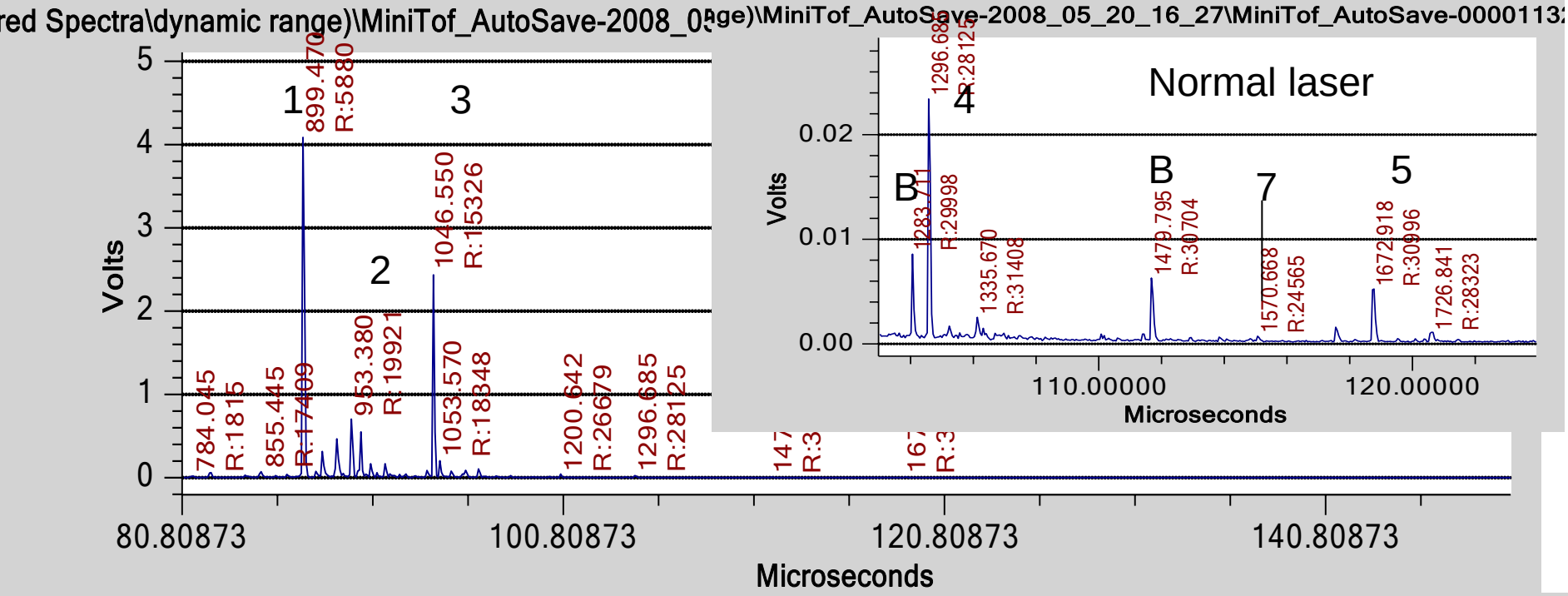
110.00000
120.00000
130.00000
140.00000



Matrix blank, 2V digitizer range, major matrix peaks off-scale

Test Mixture for Determining Dynamic Range

Peptide	Conc. (fmole/μL)	MH^+	No.
Angiotensin Fragment 1-7	10,000	899.47	1
Angiotensin II Acetate	1,000	1046.54	3
des-Pro^2 Bradykinin	100	963.51	2
Angiotensin I	2	1296.69	4
Neurotensin	1	1672.92	5
Glu-1 Fibrinopeptide B (synthetic)	0.2	1570.67	7
ACTH (18-39)	0.01	2465.20	6
BSA digest	0.1-1	1479.79	B
		927.49	
		1283.71	
		1639.94, etc	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ge)\MiniTof_AutoSave-2008_05_20_16_27\MiniTof_AutoSave-0000113
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100.80873
120.80873
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2
3
899.4700 R:5880
1046.550 R:15326
953.380 R:19921
784.045 R:1815
855.445 R:17409
1053.570 R:18348
1200.642 R:26679
1296.685 R:28125
Normal laser
4
B
7
5
1283.711 R:29998
1335.670 R:31408
1479.795 R:30704
1570.668 R:24565
1672.918 R:30996
1726.841 R:28323
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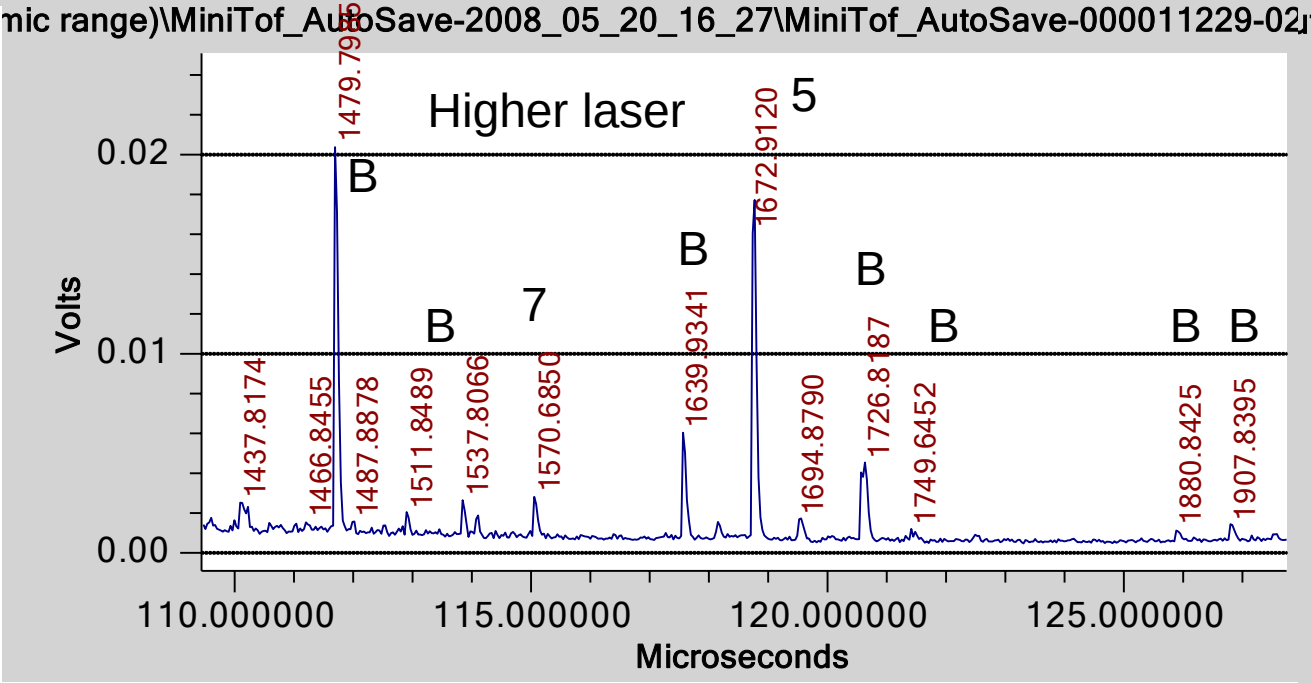
mic range)\MiniTof_AutoSave-2008_05_20_16_27\MiniTof_AutoSave-000011229-02
Higher laser
Volts
Microseconds
110.000000
115.000000
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125.000000
1479.7985
1672.9120
5
7
B
1437.8174
1466.8455
1487.8878
1511.8489
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1570.6850
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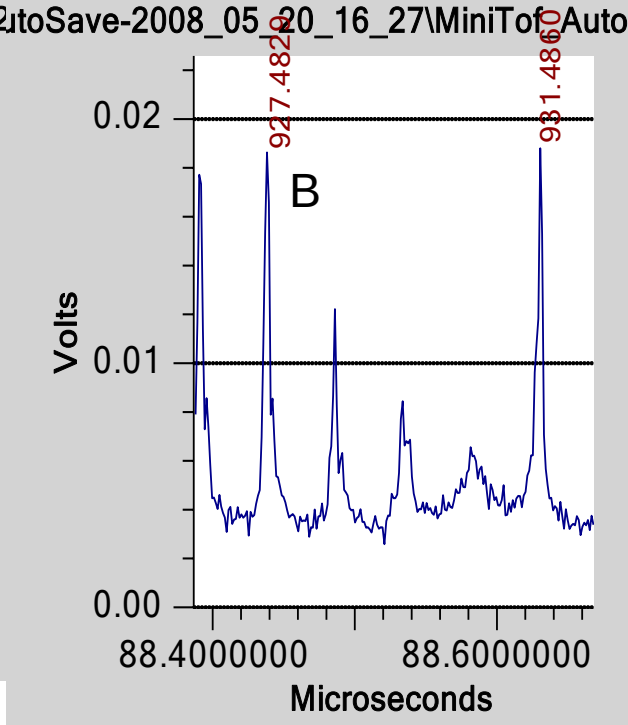
1907.8395


utoSave-2008_05_20_16_27\MiniTof_Auto
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931.4860
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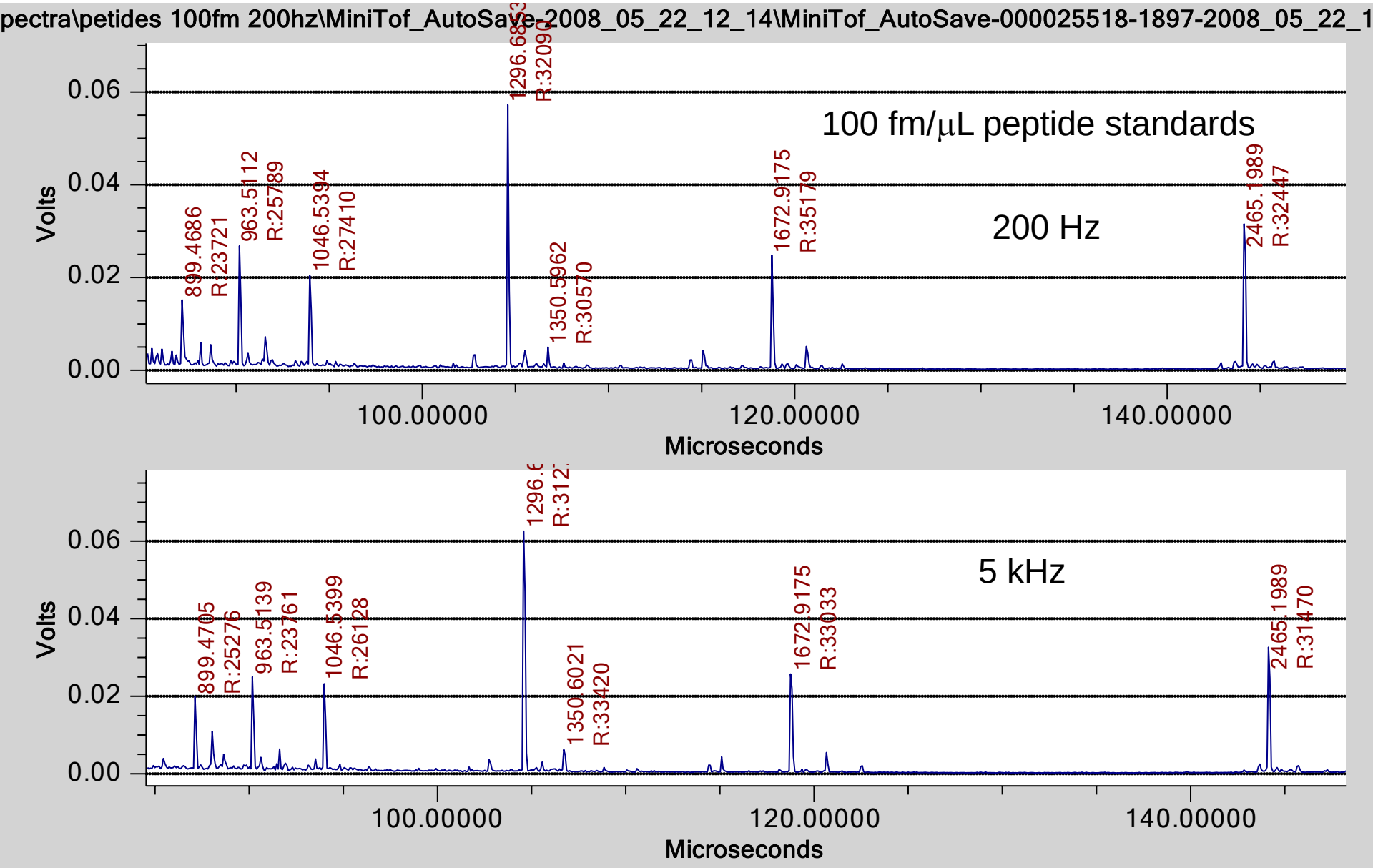

No detectable effect of laser rate on spectrum quality.

Conclusion and Future

- Present status
  - Resolving power > 30,000 for peptides
  - Mass error < 2 ppm RMS over entire sample plate
  - Detection limit ~1 attomole/μL
  - Dynamic range ~10^5

  limited by chemical noise
  - Performance independent of laser rate (to 5 kHz)
- Future goals
  - Resolving power >100,000
  - Mass error < 1 ppm RMS
  - 10x improvement in detection limit and dyanamic range
  - Isotopic resolution of proteins to 30,000 Da
  - Multiplexed MS-MS with >4000 resolving power for precursor selection and very high sensitivity and throughput
  - Instruments designed for specific applications, e.g. isotope ratios at ppt level


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Marvin
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Chemist
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Kevin
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