Quantitative MALDI-TOF for Clinical Applications

Outline

- TOF-MS for clinical applications and for research
- Why is MALDI-TOF not quantitative?
- How do we make it quantitative?
- Some example of quantitative MALDI.

Major advances for practical MALDI-TOF for quantitative and clinical applications

- Lasers
- Digital electronics
- Ion detectors
- Ion optics
- Delayed extraction/time lag focusing
- Motion control
- DAC control of electronics
- Software, software, software

Lasers

Nitrogen
337 nm,
10-50 Hz
50-500 shots/spectrum
large spot size
short life (10 million shots)

OLD

Solid State, tripled YAG/YLF
349 or 355 nm
1-10 kHz
1000-1,000,000 shots/spectrum
small spot size (20-100 μm)
long life (100 billion shots)

NEW

Digitizers

OLD

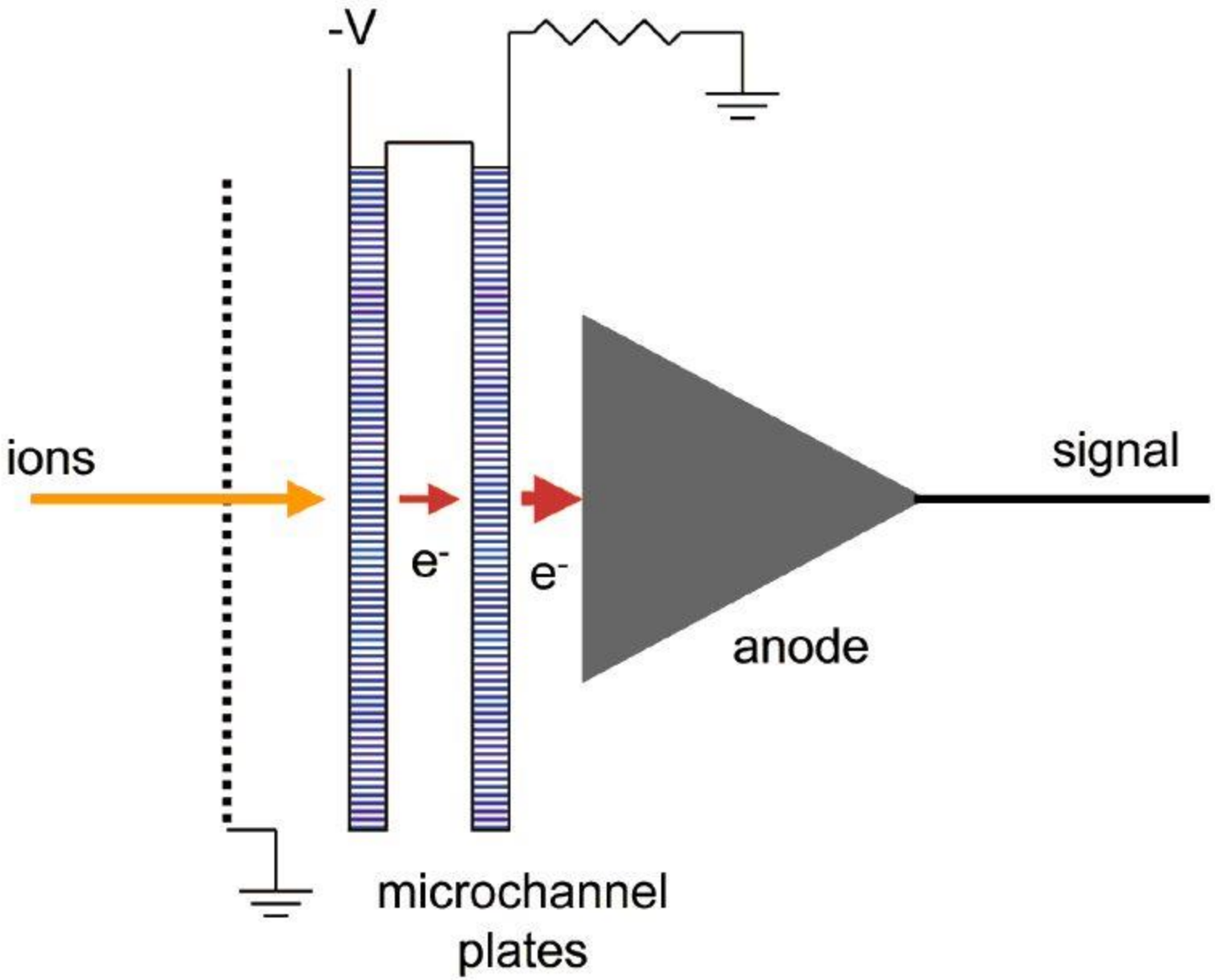
TDC (time-digital convertor) <1 ion/channel/shot

Transient Digitizer (digital oscilloscope)

NEW

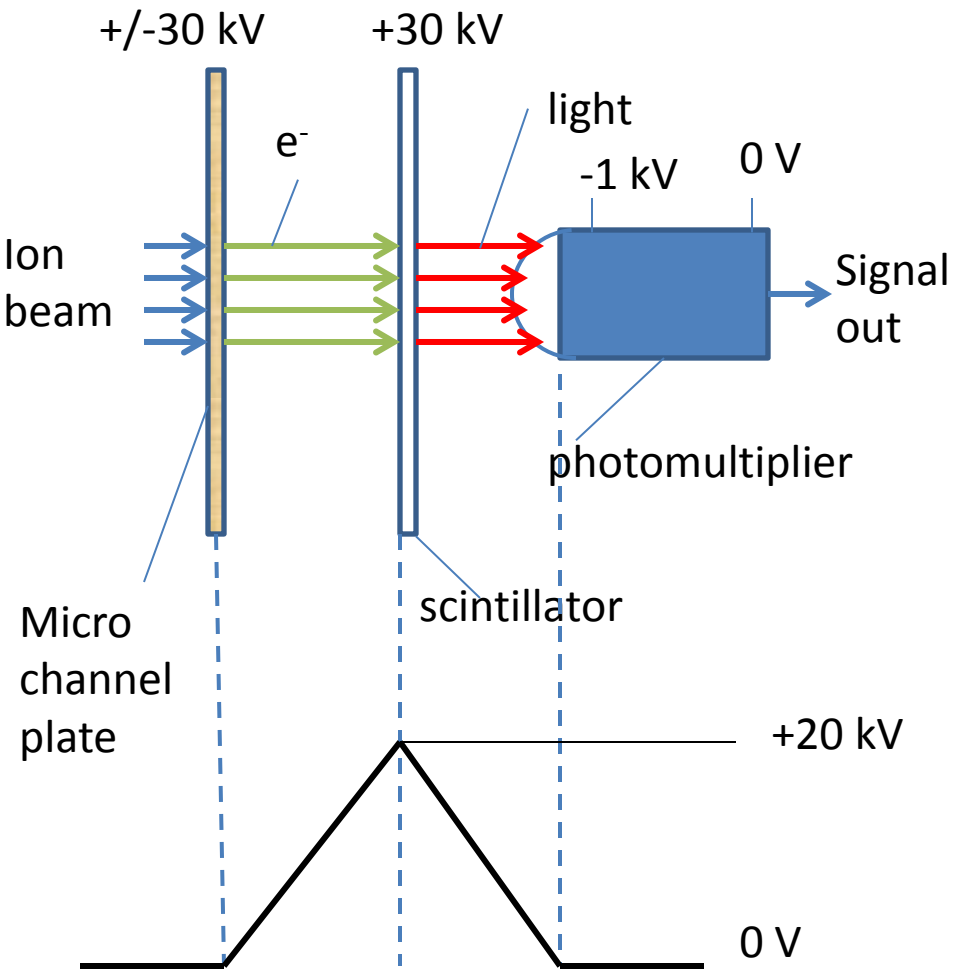
Detectors

- Dual Channel Plate
  - Flat input surface
  - Very fast
  - Channels saturated by low mass ions
- Discrete Dynode
- Hybrid
- Exotic


-V
ions
e-
e-
signal
anode
microchannel
plates

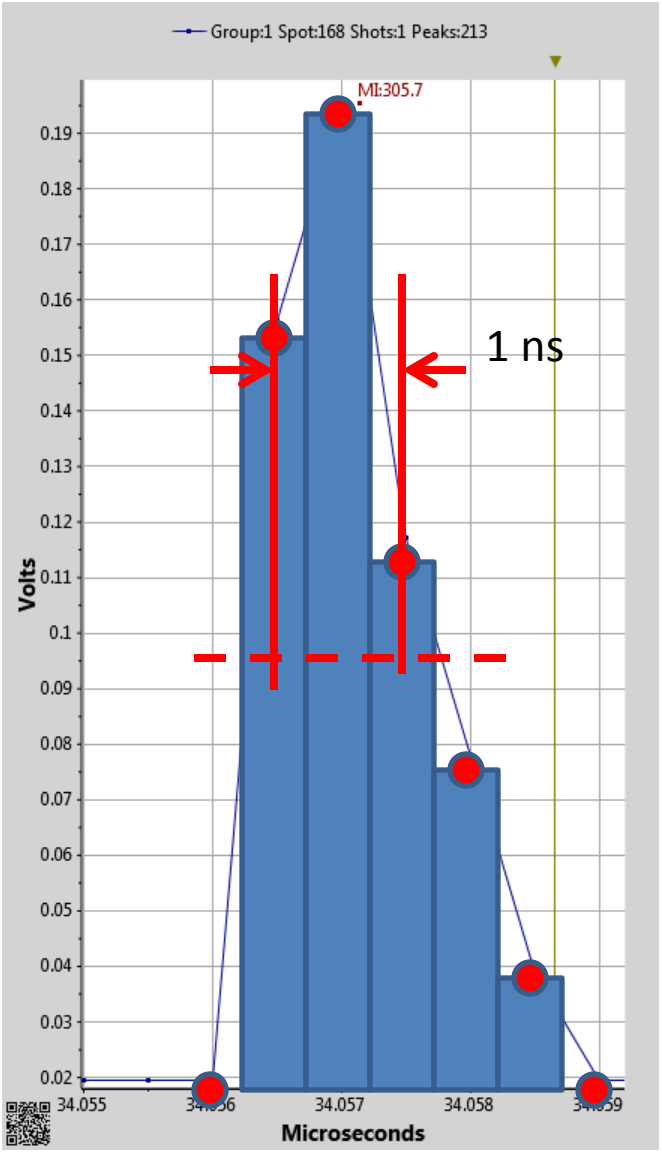
ETP 500 ps
Discrete dynode
Magnetic multiplier

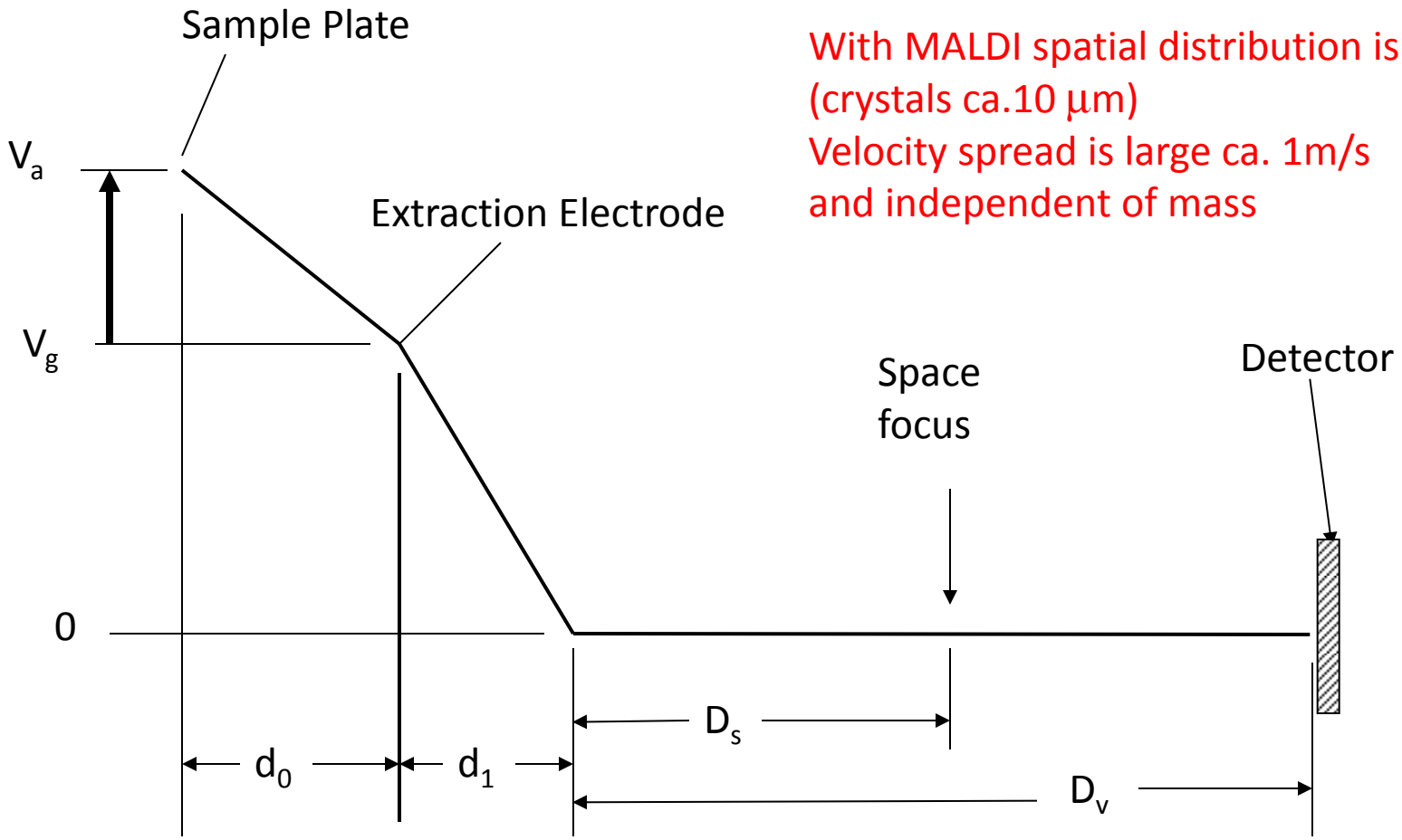
Photonis detector



Potential diagram for linear detector

Typical single ion pulse with fast scintillator

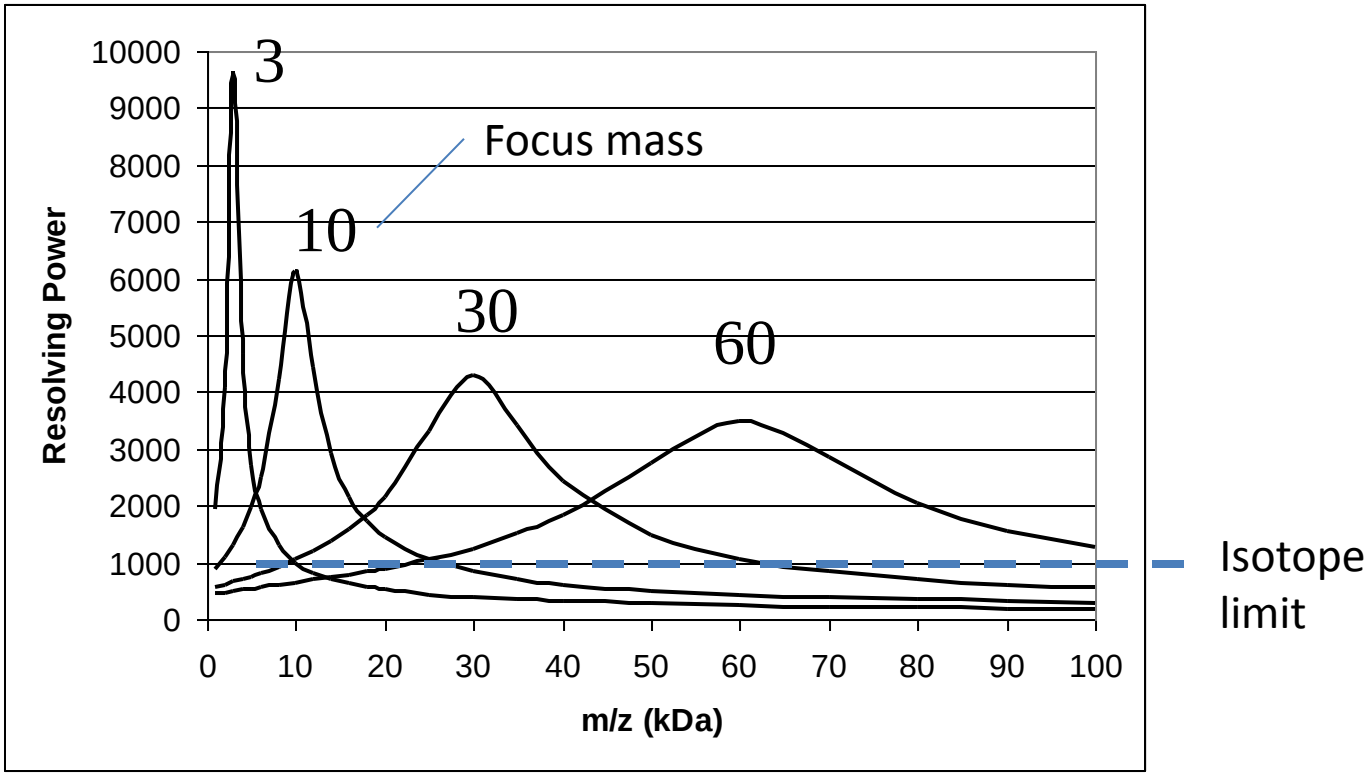




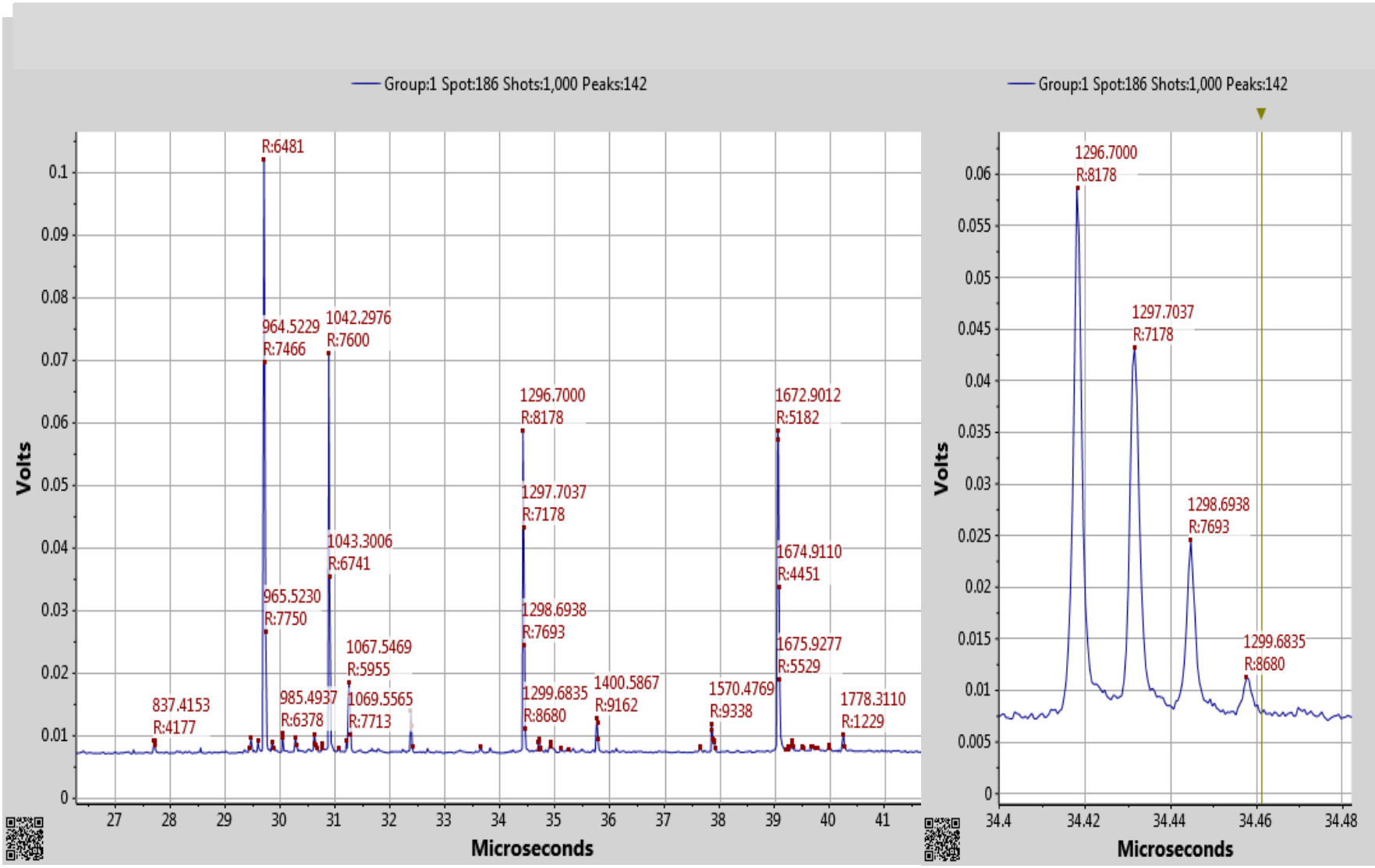
With MALDI spatial distribution is small (crystals ca.10 µm)
Velocity spread is large ca. 1m/s and independent of mass

Brown &Lennon 1992, Delayed extraction

Wiley &Mclaren 1953, Time lag focusing



Maximum Resolving Power for Linear Analyzer.



Peptide mass spectrum for modern optimized linear analyzer with effective length of 1.6 m operating at 10 kV. M. L. Vestal and K. Hayden, Int. J. Mass Spectrometry **268**, 83-92 (2007).

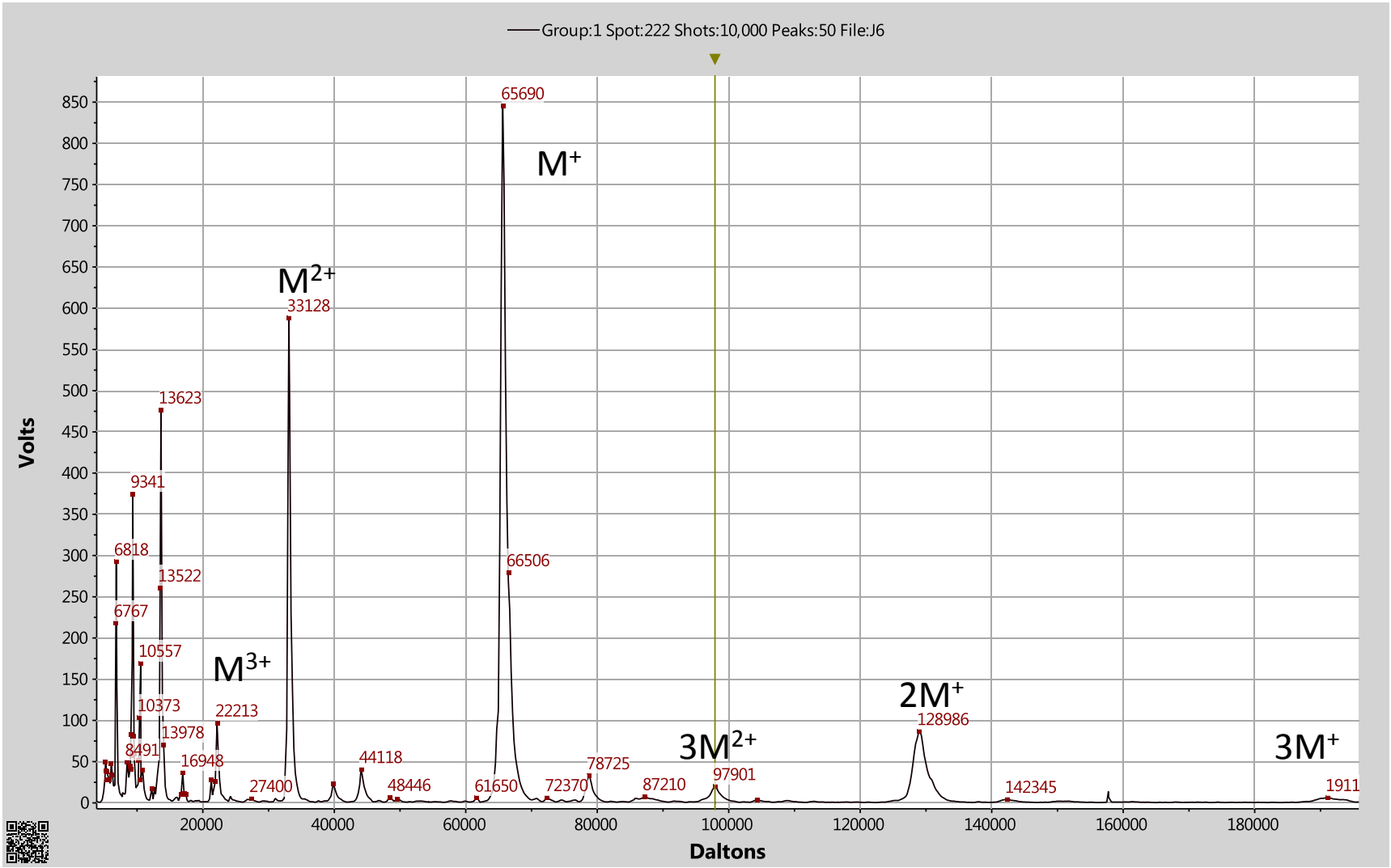
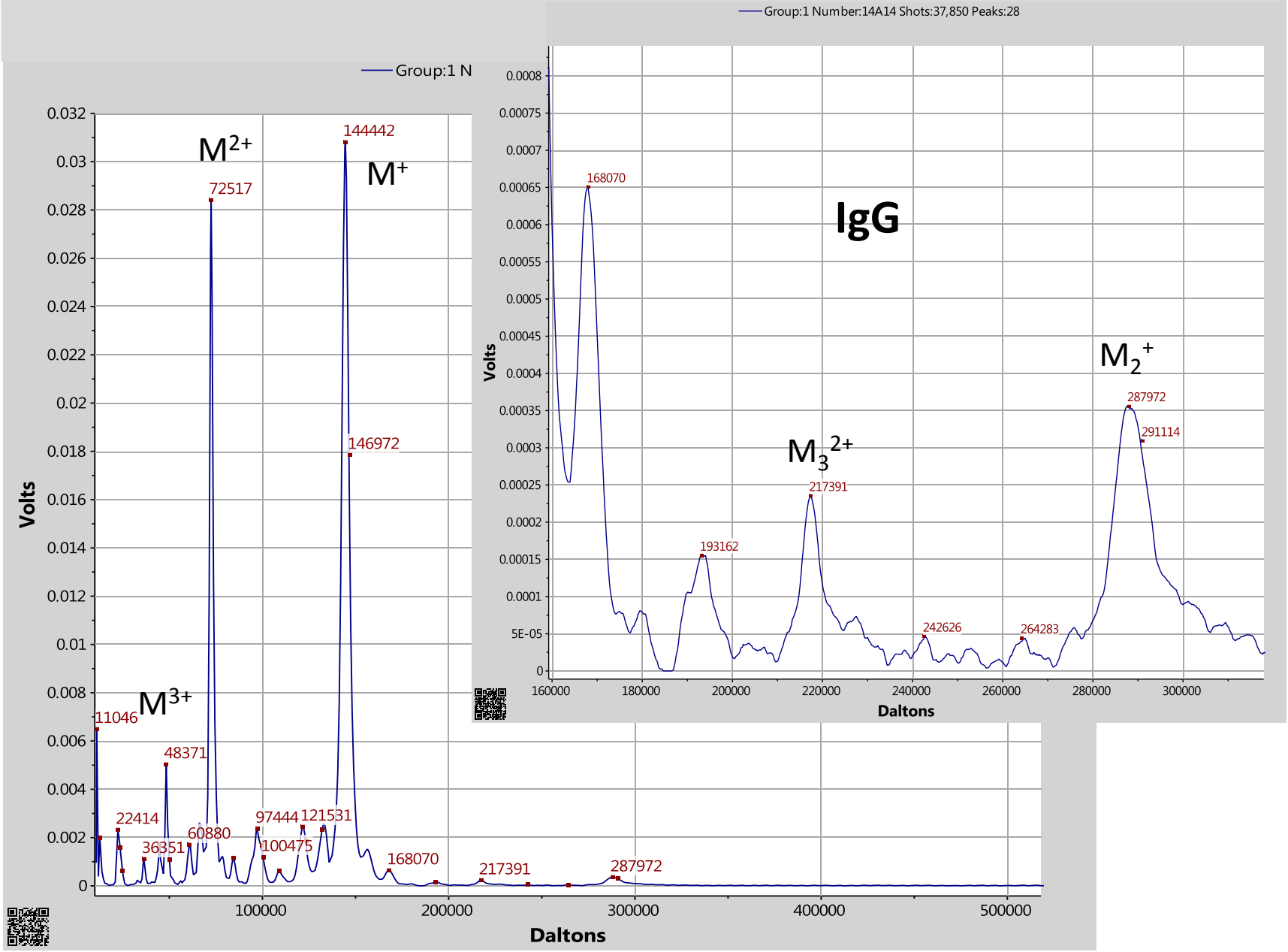
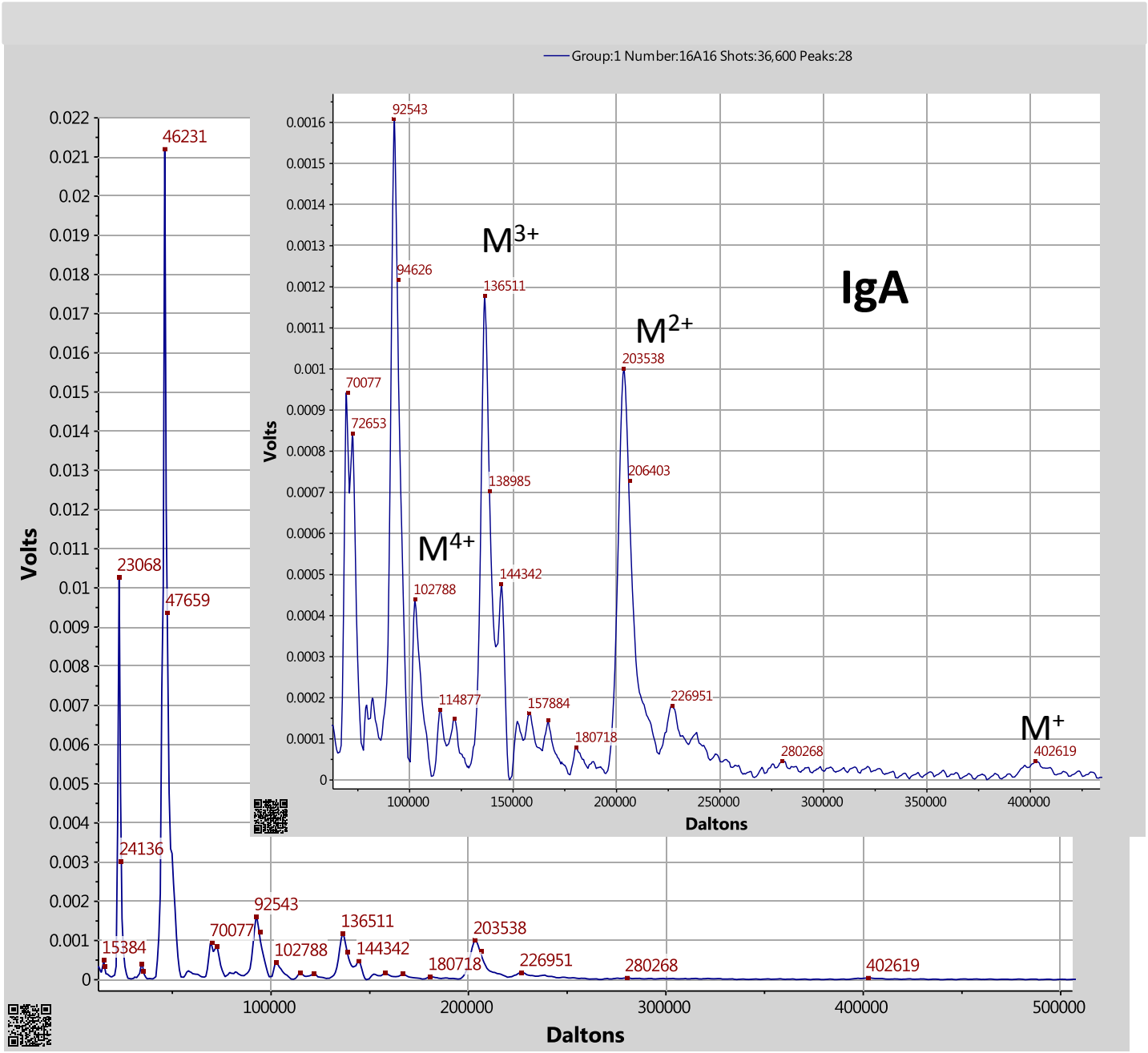


Fig. 9 BSA (1 picomole/µl) in sinapinic acid matrix 10,000 laser shots in 2 s.


Group:1 Number:14A14 Shots:37,850 Peaks:28
Group:1 N
IgG
M2+
M+
M3+
M2+
M3 2+
144442
72517
146972
11046
48371
22414
36351
60880
97444
121531
100475
168070
217391
287972
168070
193162
217391
242626
264283
287972
291114
Volts
Daltons


Group:1 Number:16A16 Shots:36,600 Peaks:28
IgA
M3+
M2+
M4+
M+
Volts
Daltons
46231
23068
47659
24136
15384
92543
70077
102788
136511
144342
180718
203538
226951
280268
402619
94626
72653
138985
206403
114877
157884

Linear MALDI-TOF

- Only mass spectrometer providing high sensitivity for singly charged high mass ions
- Resolving power 500-1000 over wide range is routine
- Normalization to TIC 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 <50 ppm across the plate over the full mass range with single peak automatic calibration
- Dynamic range up to100,000

Instruments for Clinical MALDI

- Linear MALDI-TOF preferred for most clinical applications
  - Simple, reliable, robust, and very sensitive over wide mass range
  - Reproducible spectra with wide dynamic range
  - Adequate mass resolving for higher mass proteins and oligos
  - Resolving power and mass accuracy may be insufficient for some applications to peptides and small molecules
- Reflector MALDI-TOF provides higher resolving power and mass accuracy
  - Allows identification by mass fingerprinting at low mass (e.g tryptic digests)
  - Provides accurate mass for input to MS-MS identification
- MALDI MS-MS

Why is MALDI-TOF not Quantitative?

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337 nm,
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OLD

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NEW

Digitizers

OLD

TDC (time-digital convertor) <1 ion/channel/shot

Transient Digitizer (digital oscilloscope)

NEW

Introducing

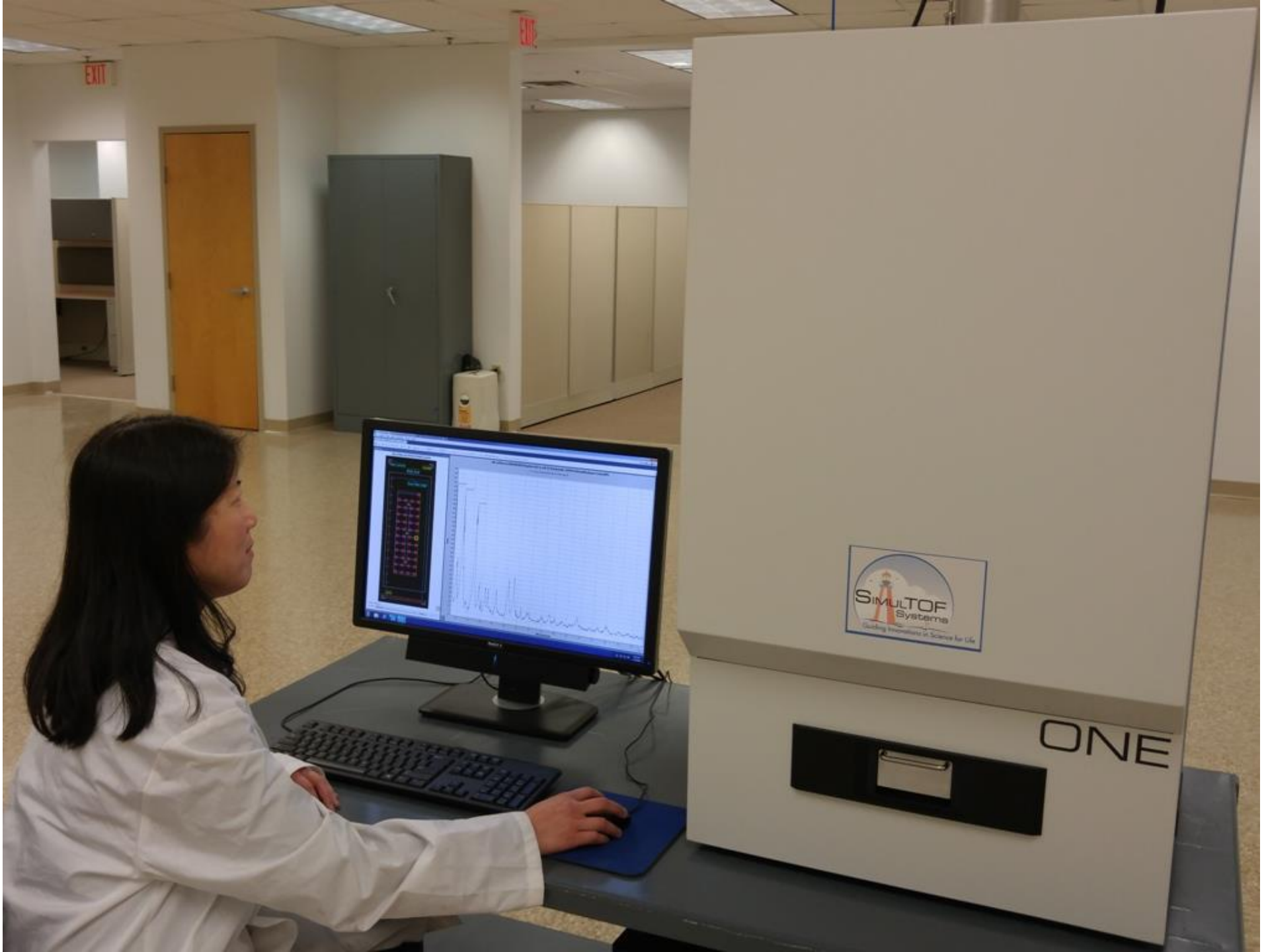
SimulTOF ONE MALDI-TOF

Designed to make MALDI-TOF QUANTITATIVE!!!

Visit booth 20
or
Website: SimulTOF.com
for details and demos

SimulTOF ONE MALDI-TOF for quantitative and clinical applications optimizes all of the elements employing state-of-the-art technology

- Lasers
- Digital electronics
- Ion detectors
- Ion optics
- Delayed extraction/time lag focusing
- Motion control
- DAC control of electronics
- Software, software, software


EXIT
EXIT
SIMULTOF
Systems
ONE

Now you can have your personal high performance mass spectrometer on your desk at a price you can afford!!!

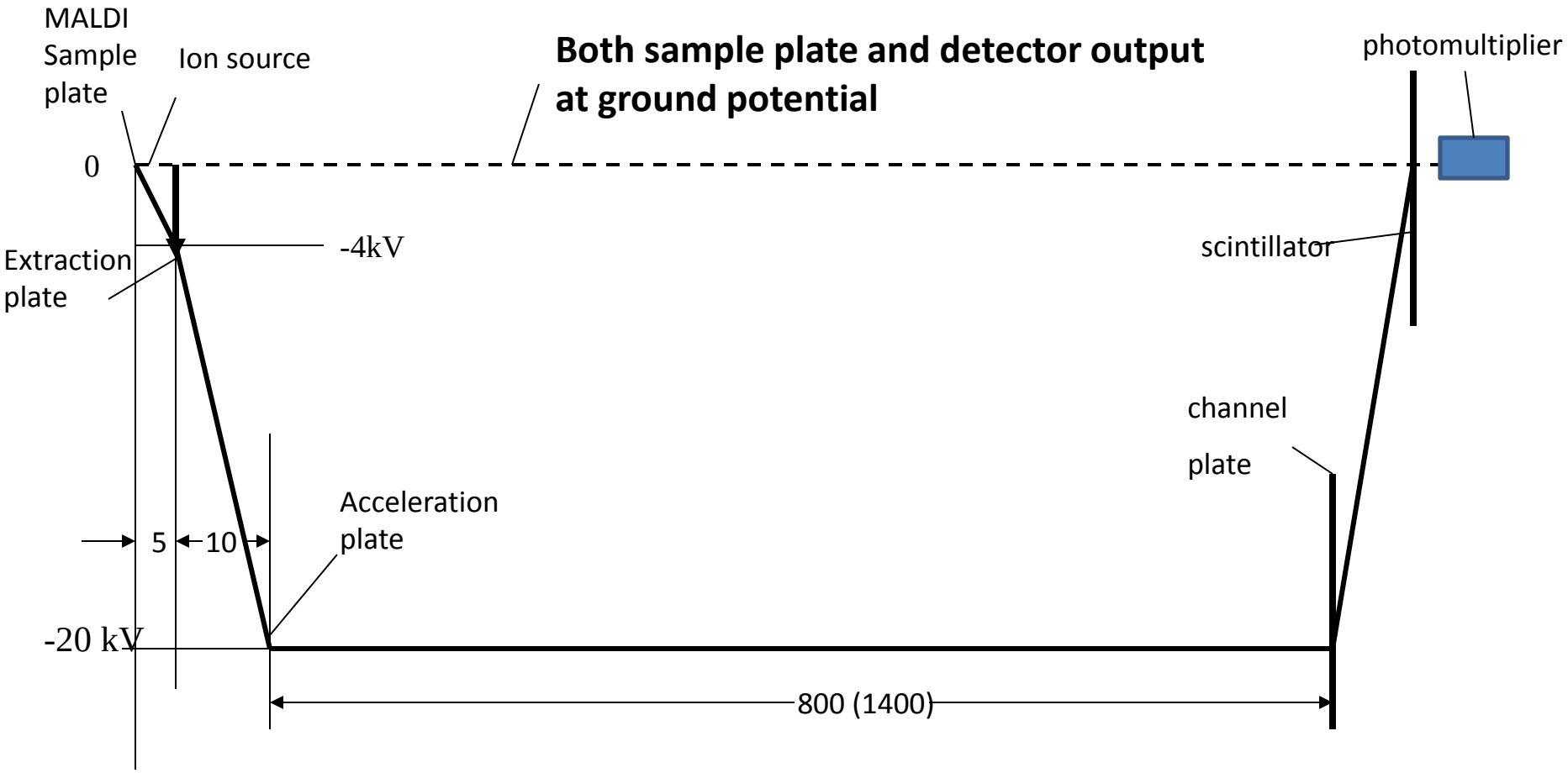
And all of your colleagues can share it.

Up to five users on their personal computers at no additional cost

- Highest performance available from any linear MALDI-TOF MS over full mass range to 1,000,000 Da.
- Smallest footprint of any linear MALDI-TOF
- High performance hybrid detector provides excellent mass range, speed and dynamic range.
- Up to 100 spectra/second recorded and processed
- Standard Options include 5 kHz laser with 2 GHz digitizer; bipolar ion analyzer; and very fast plate autoloader for high throughput applications.
- Technology developed and patented at SimulTOF produces robust MALDI-TOF mass spectrometers with no compromise in performance at an affordable price.

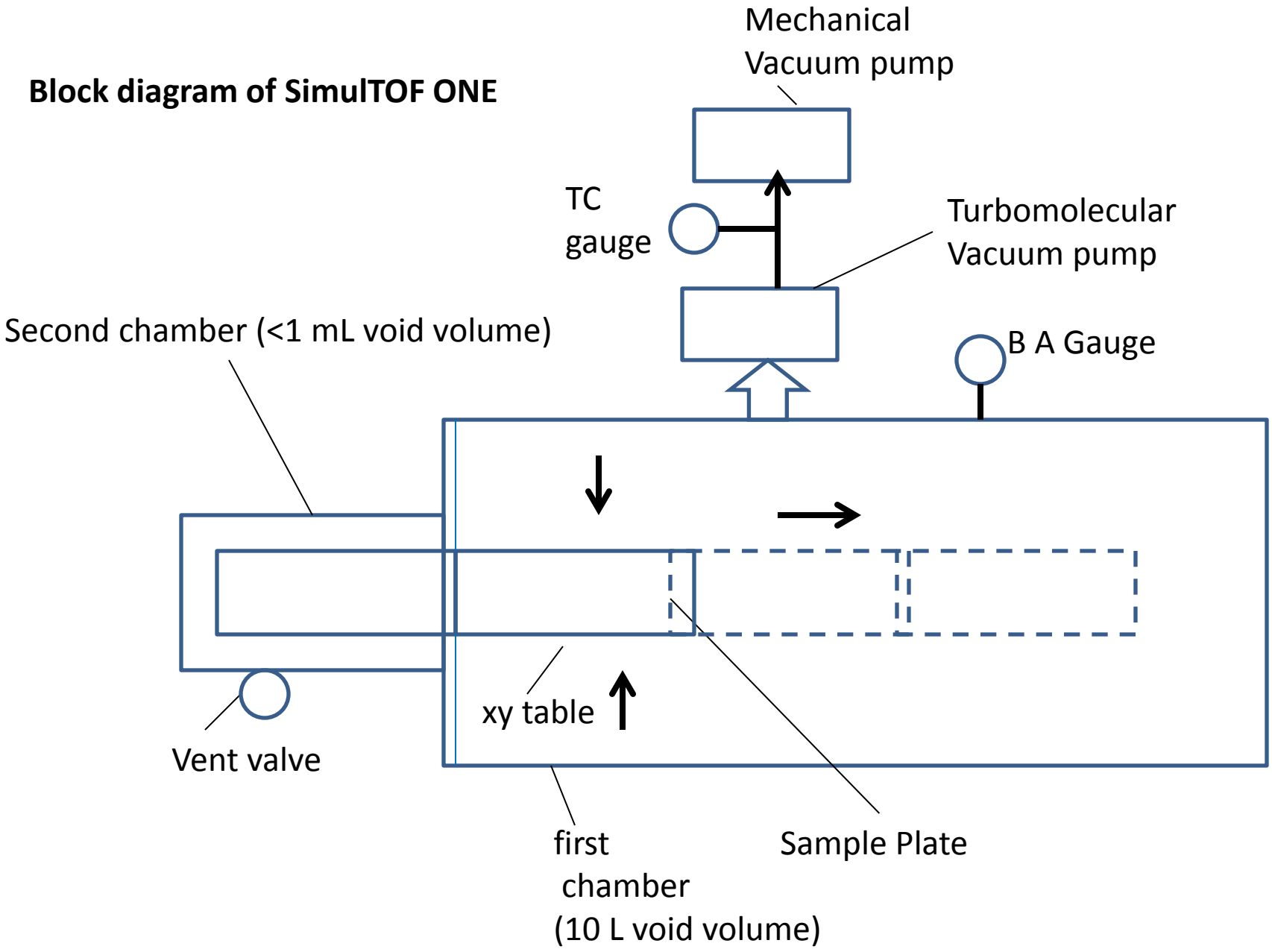
Features of SimulTOF ONE

- 20 kV ion energy and novel high speed, high mass detector provides very high sensitivity, resolving power, and accuracy over broad mass range
- Very fast sample plate exchange
- Fully automated and designed for ease-of-use by first-time users
- Intuitive software that requires only minimal training
- Up to 100 spectra/second recorded and processed
- New concepts in instrument design provide a system that is simple, reliable, and robust with only minimal preventative maintenance
- Computer controlled laser fluence
- Self-contained vacuum system
- Single 20A circuit powers complete system including computer
- No other utilities required



SimulTOF ONE Linear Analyzer

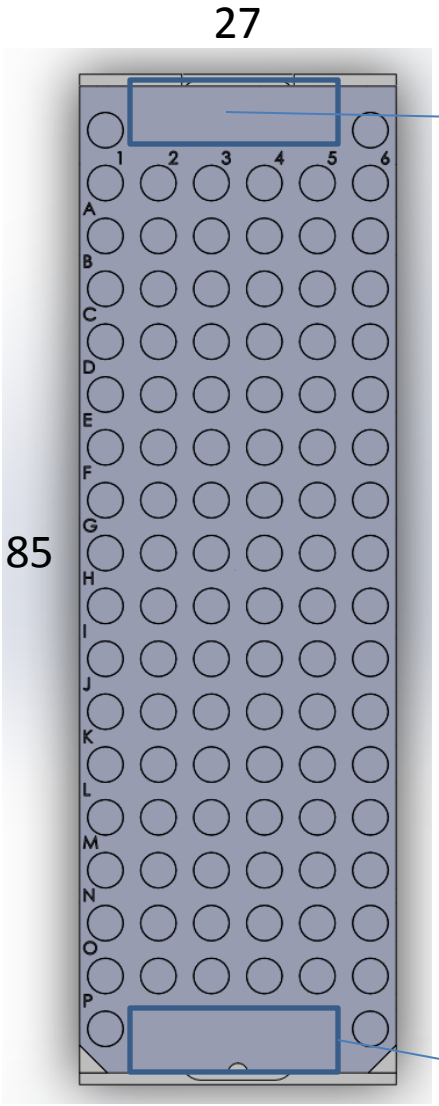
Block diagram of SimulTOF ONE



96 spots
Plus 4 cal spots

4 plates equal one
384 microtiter plate

2.6 mm dia spots



Dimensions in mm

One example of
Sample plate for
SimulTOF ONE

Reproducibility of MALDI-TOF on well-behaved sample (saliva)

Reproducibility of spectra acquired with small number of laser shots

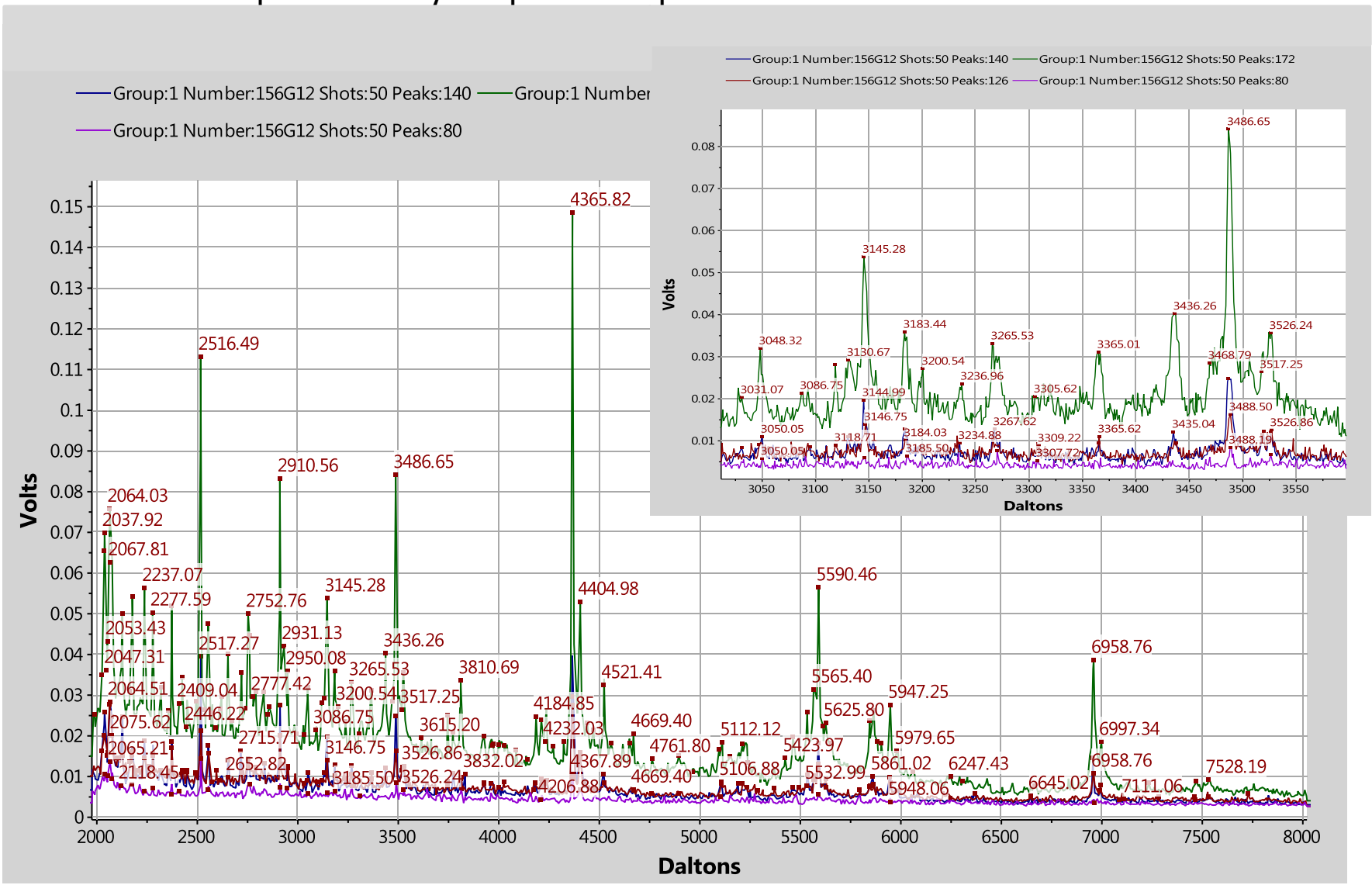


Figure 1. Comparison of saliva spectra with 50 laser shots averaged.

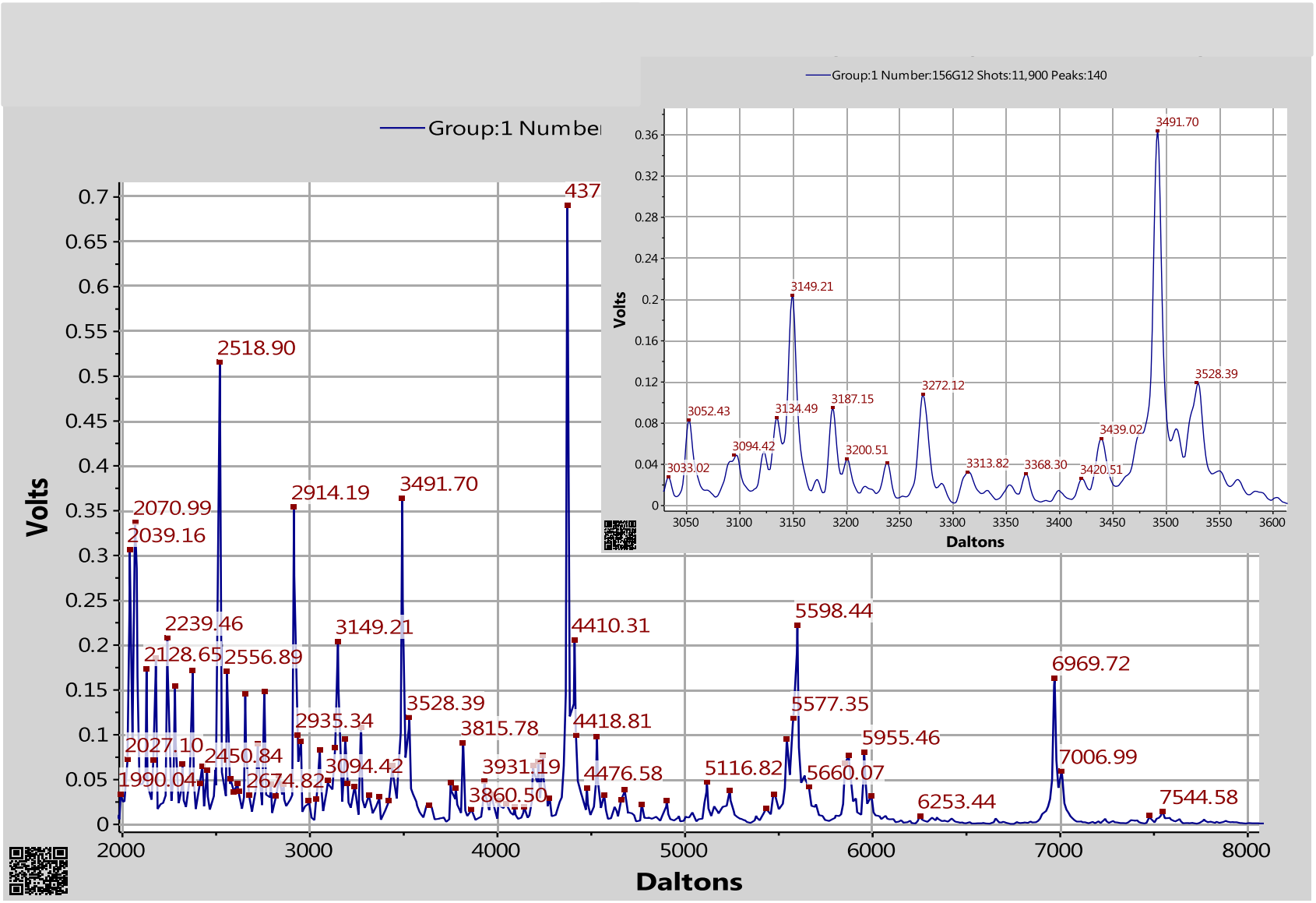


Figure 2. Saliva spectrum with **11,900** laser shots averaged.

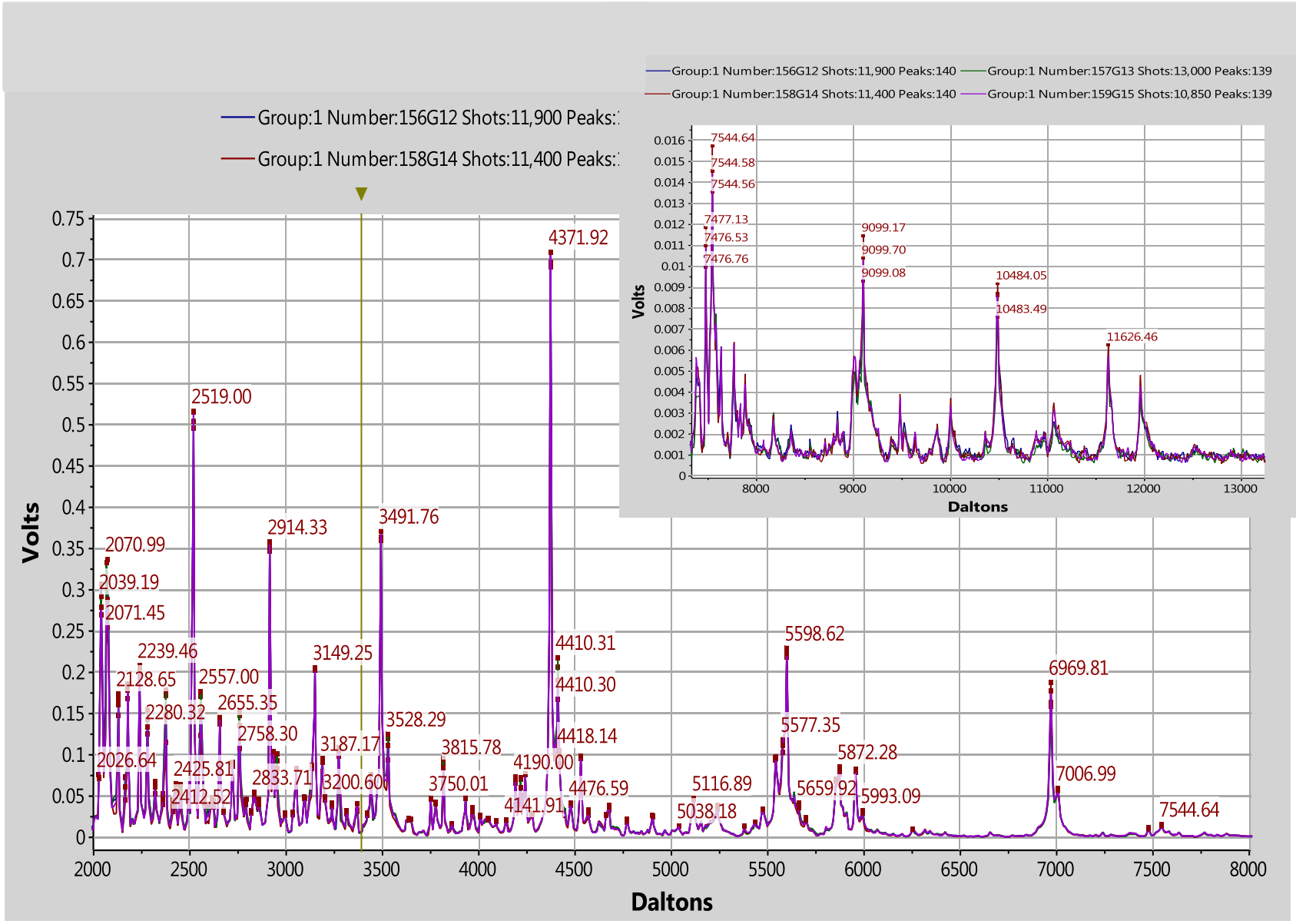


Figure 3. Comparison of Saliva spectrum from 4 spots with 11,000 laser shots averaged.

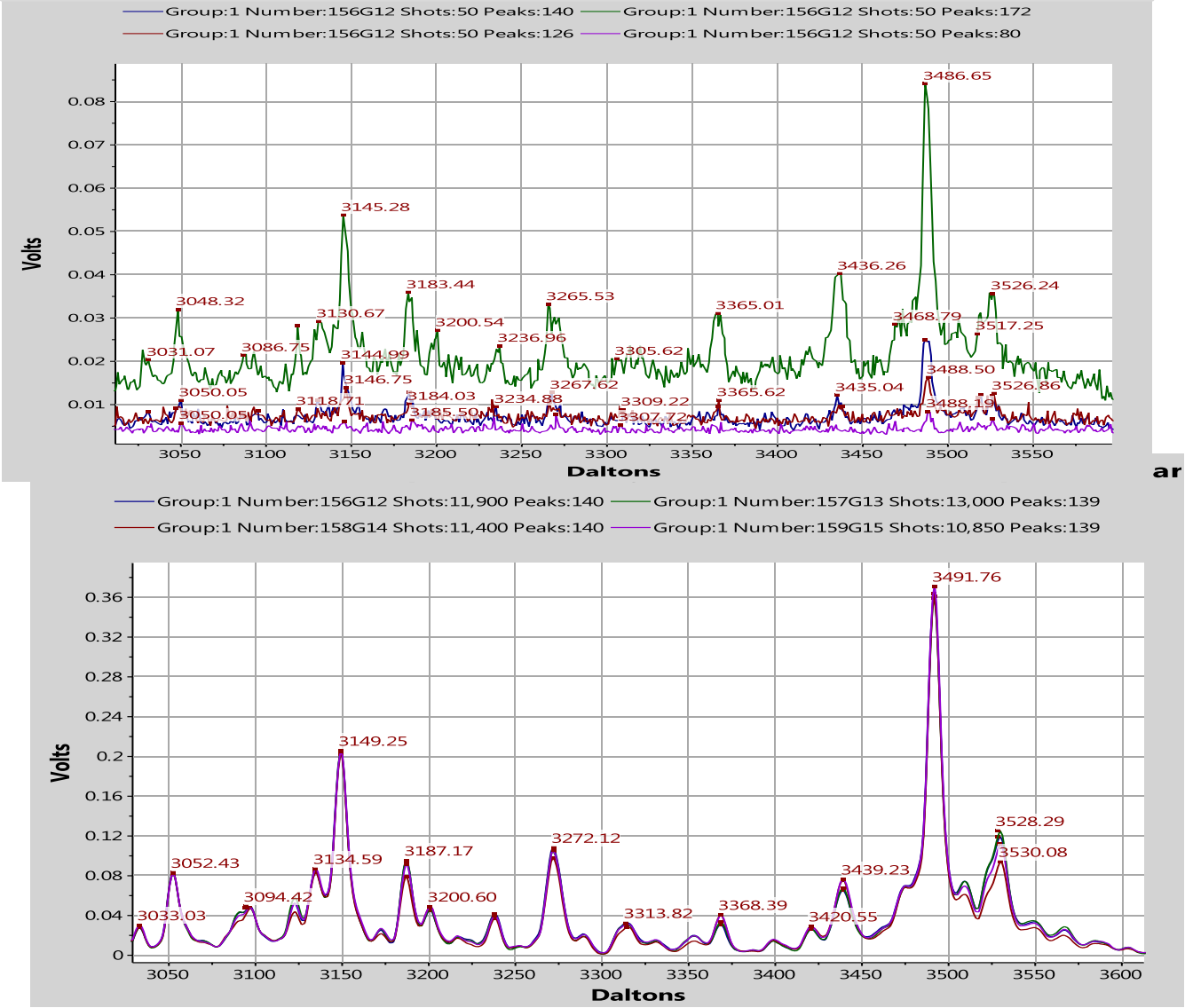
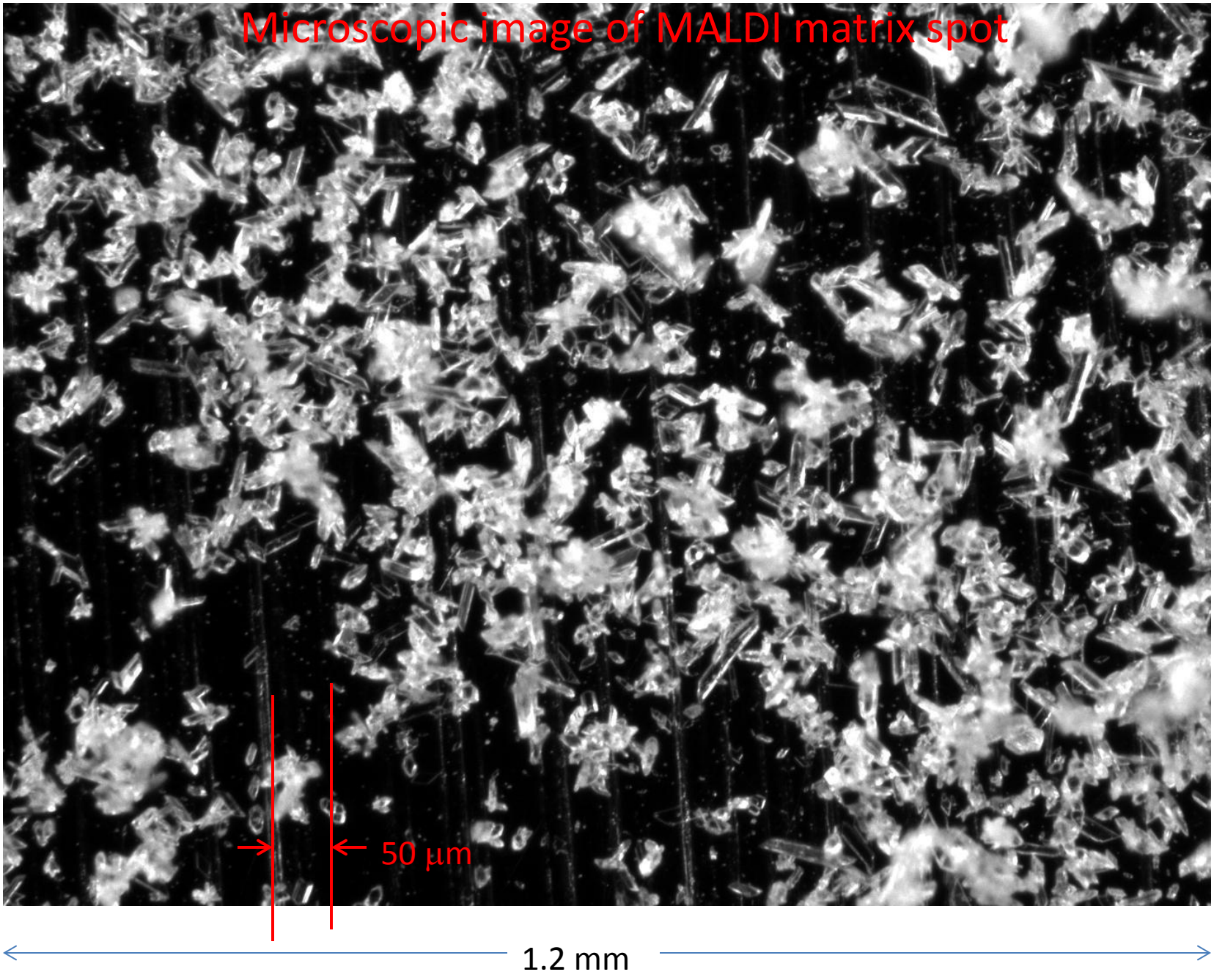


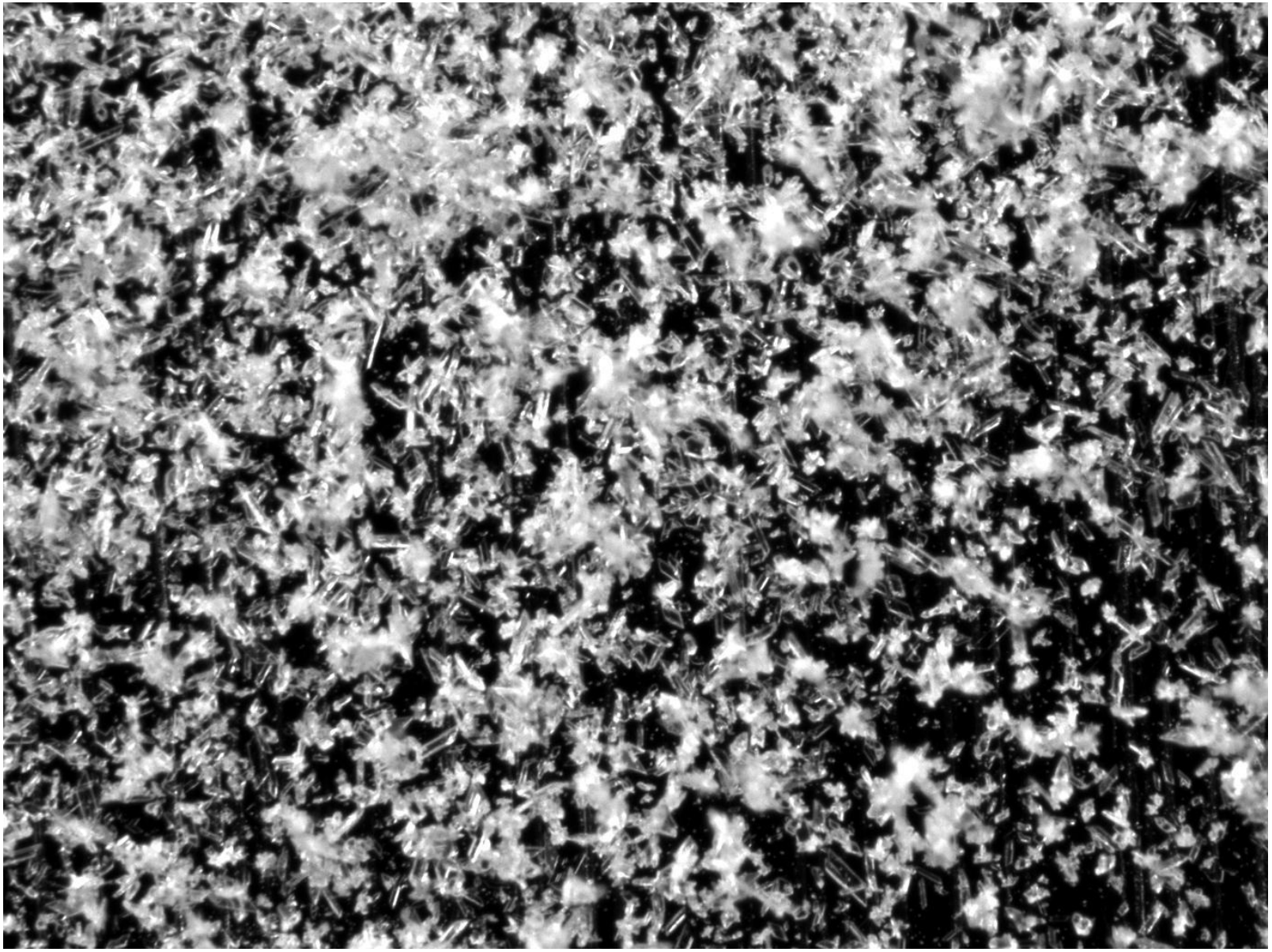
Figure 4. Comparison of 4 saliva spectra with 50 laser shots averaged vs. 4 with 11,000 laser shots over mass range 3 to 3.6 kDa


Microscopic image of MALDI matrix spot
50 µm
1.2 mm

Microscopic image of MALDI matrix spot

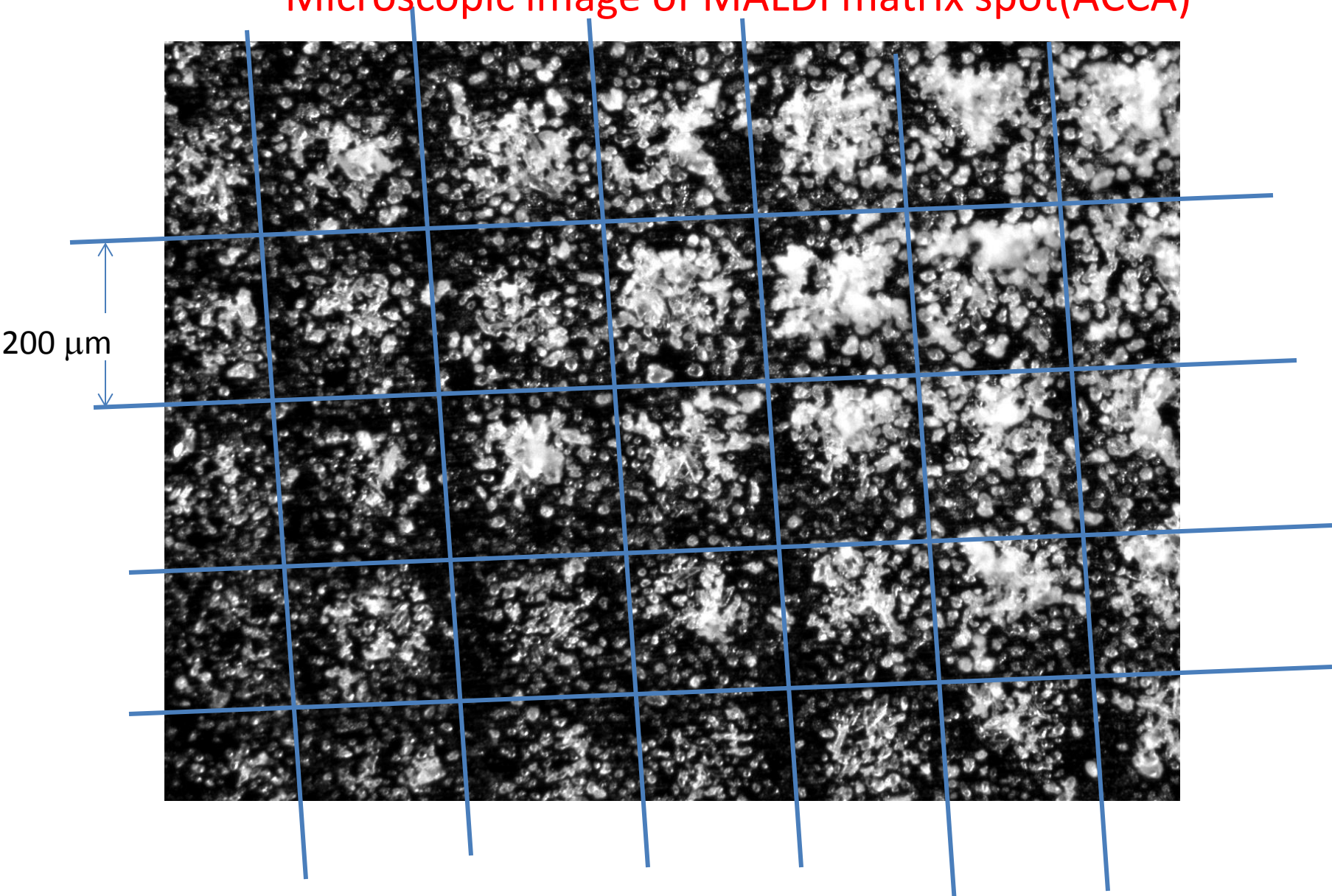
Sinapinic acid

Microscopic image of MALDI matrix spot (ACCA)



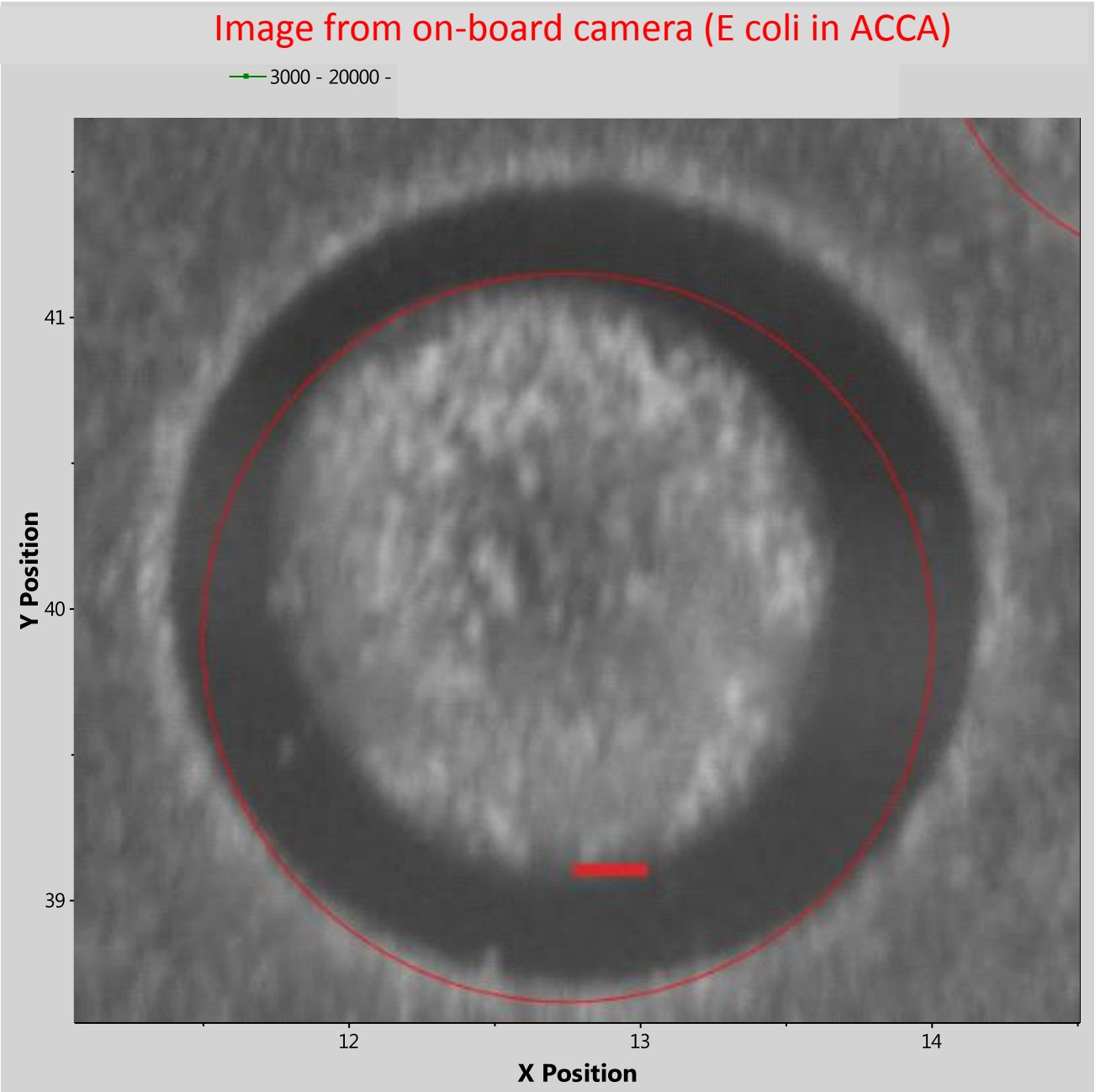
α–cyano-4-hydroxy cinnamic acid

Microscopic image of MALDI matrix spot(ACCA)



After laser raster at 200 µm spacing

Image from on-board camera (E coli in ACCA)



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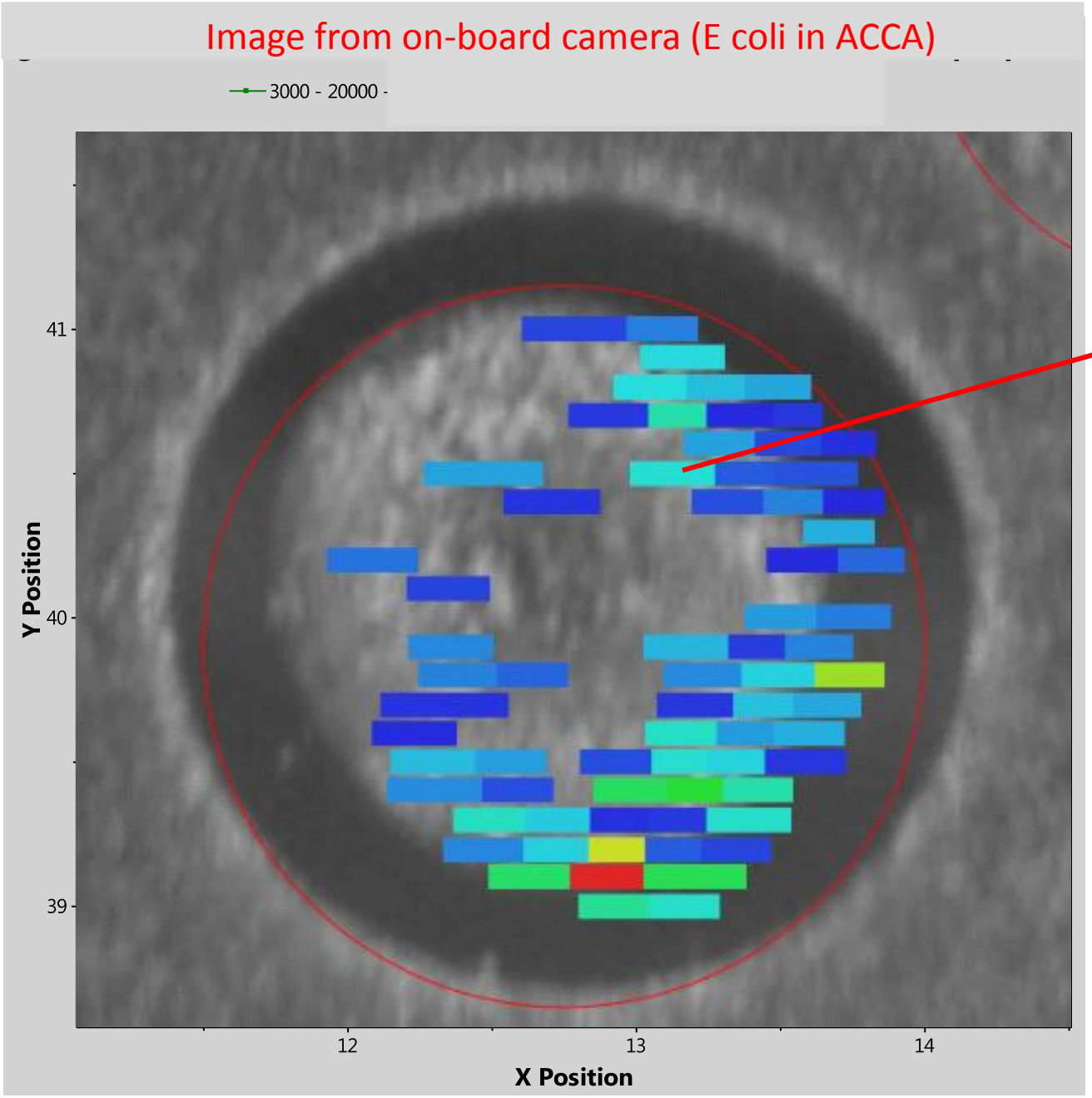

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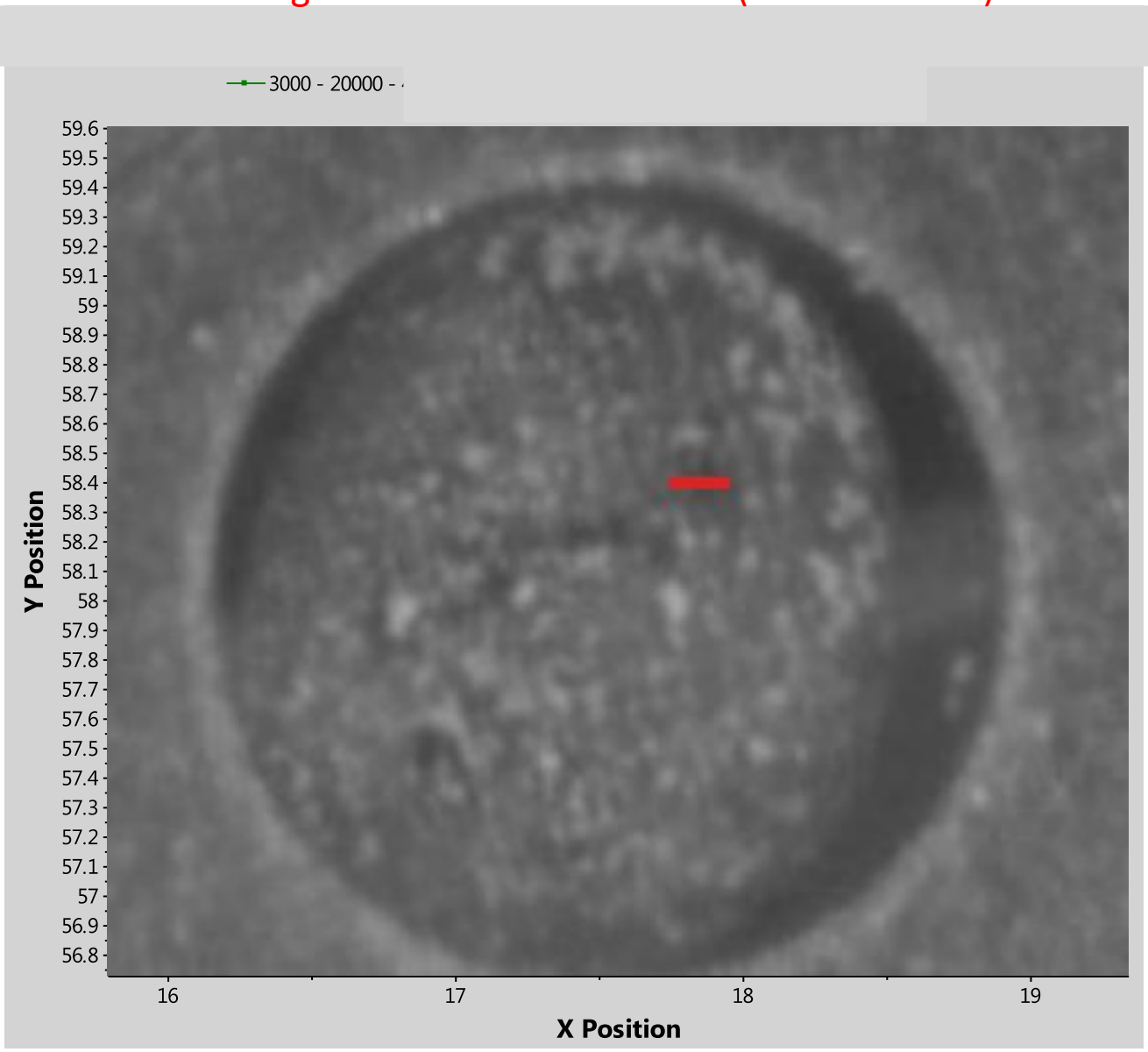
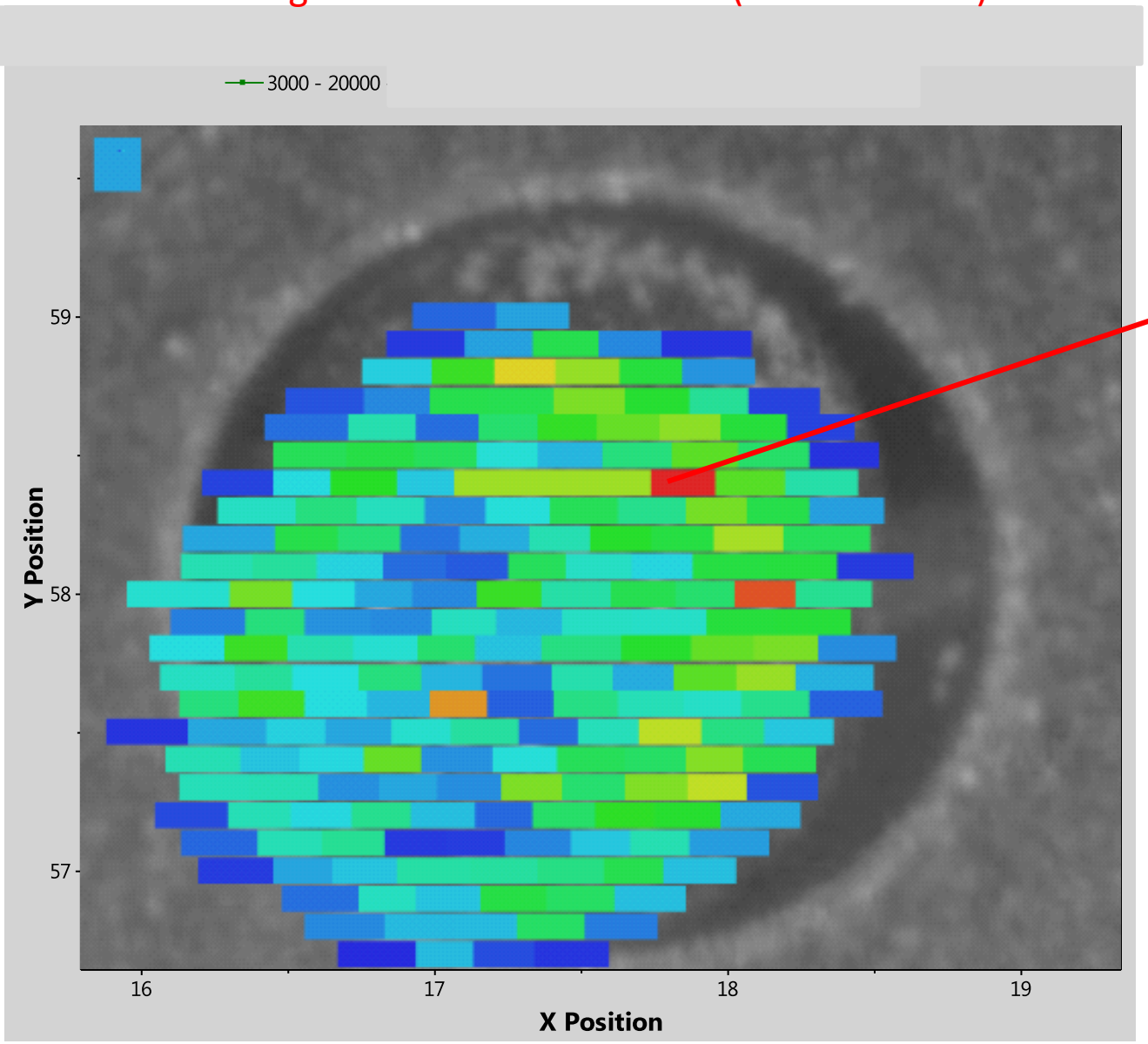
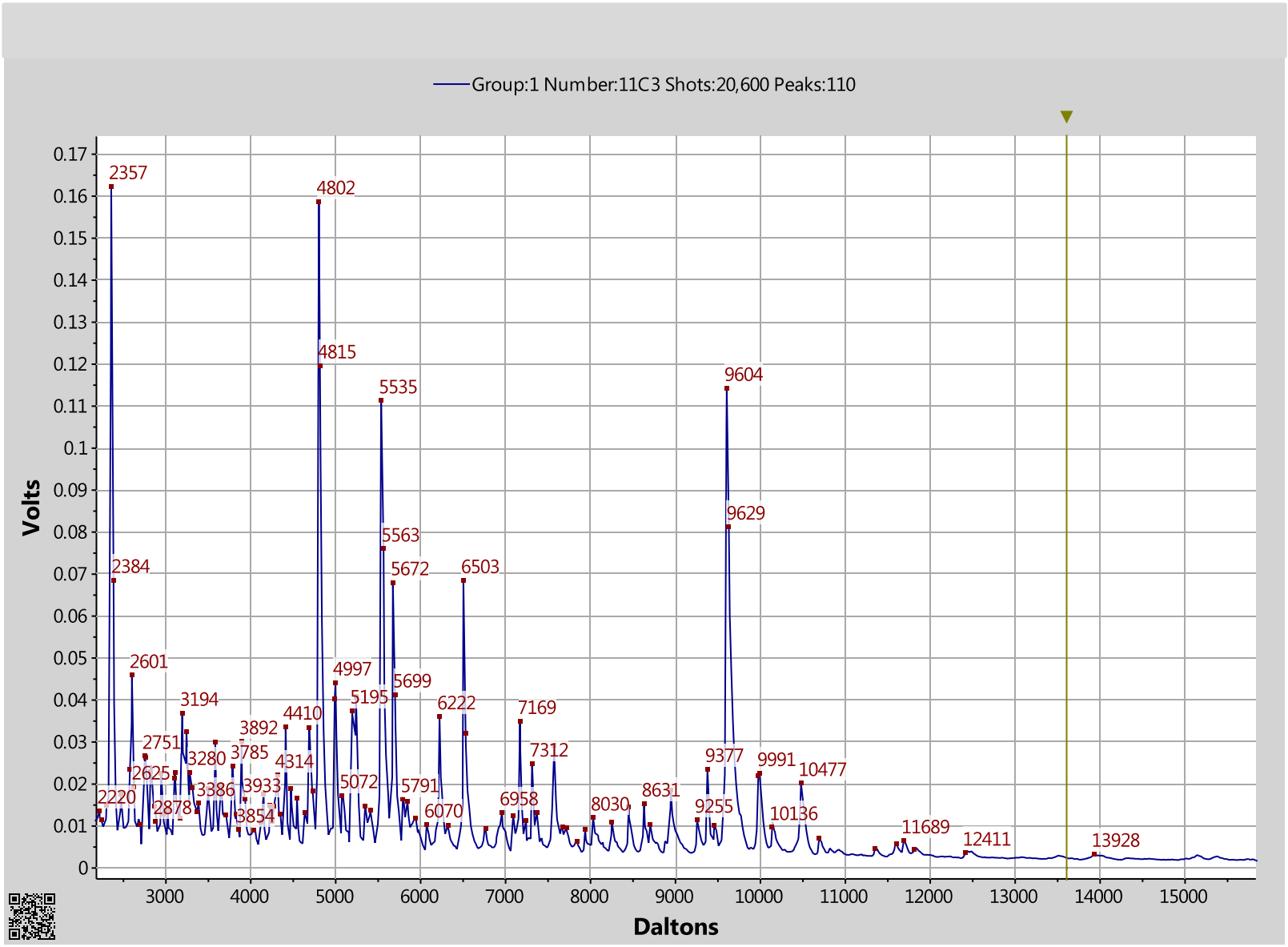
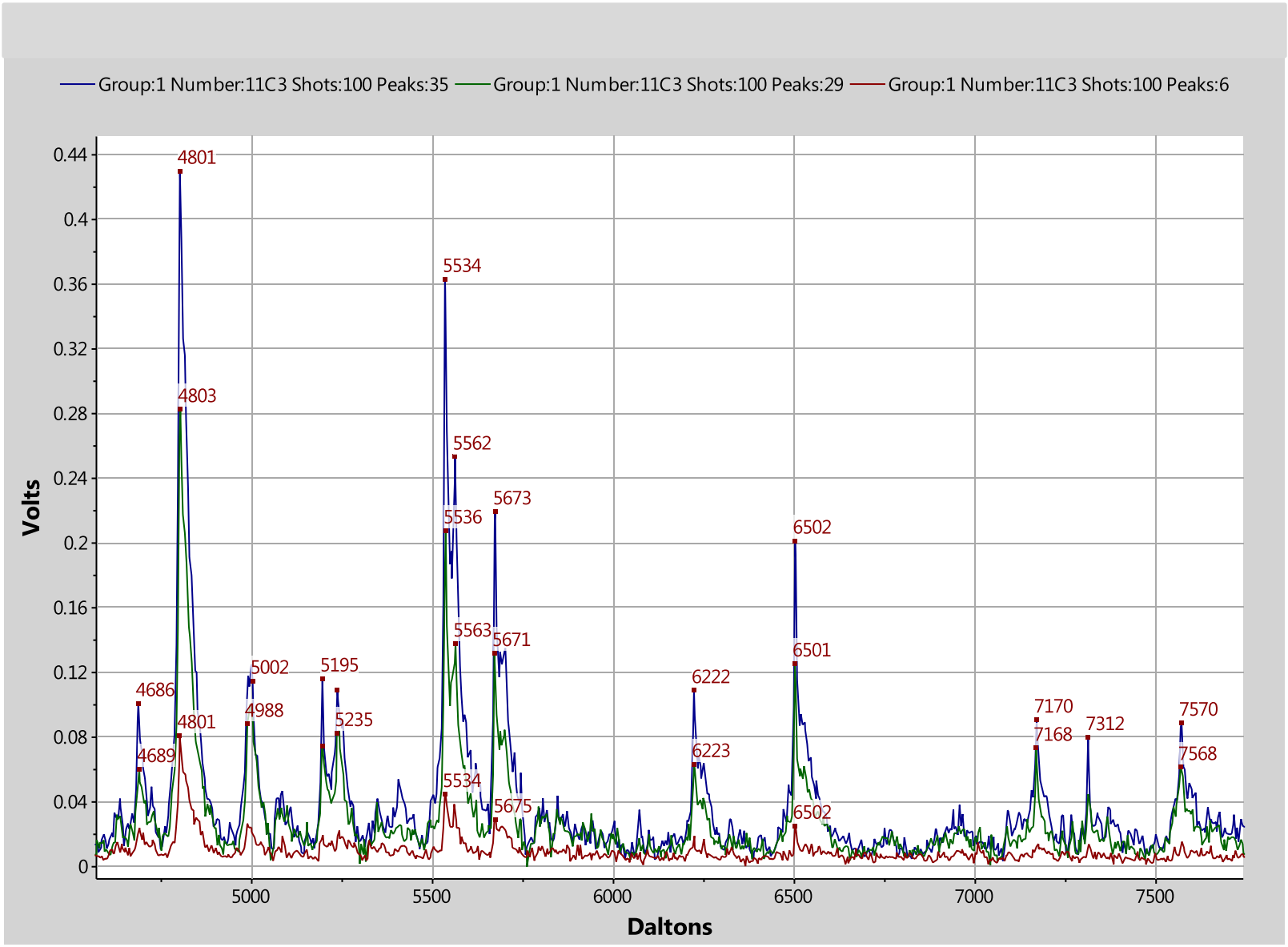


Image from on-board camera (E coli in ACCA)

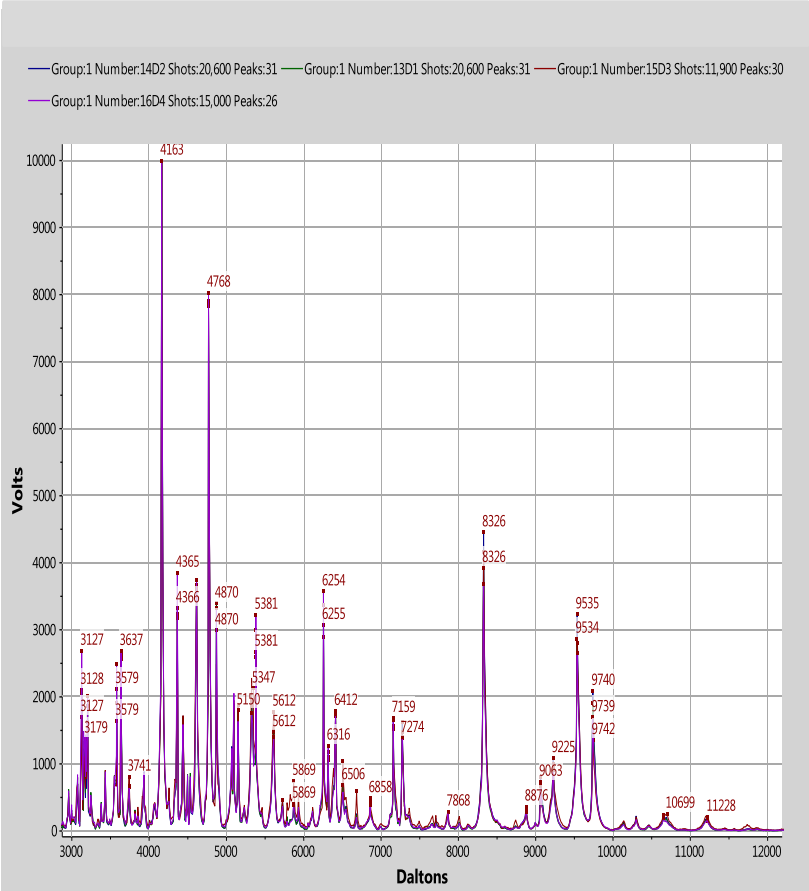




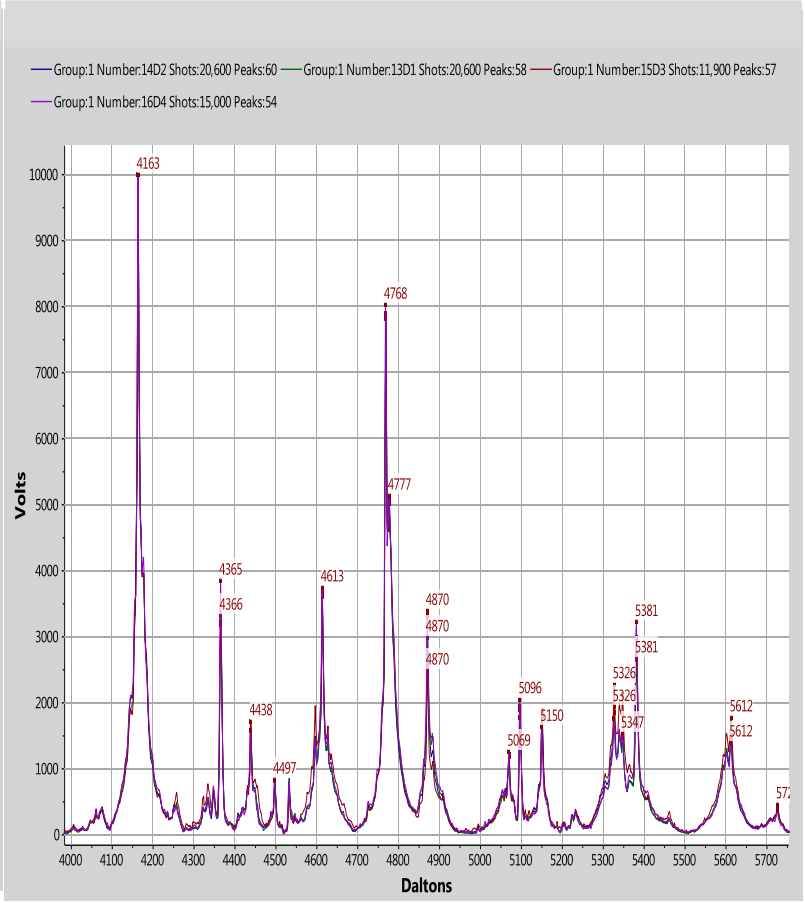
E coli mass spectrum (ACCA)



Spectra from 3 pixels on one spot



Superposition of spectra from four spots of E coli in HCCA matrix



Expanded View

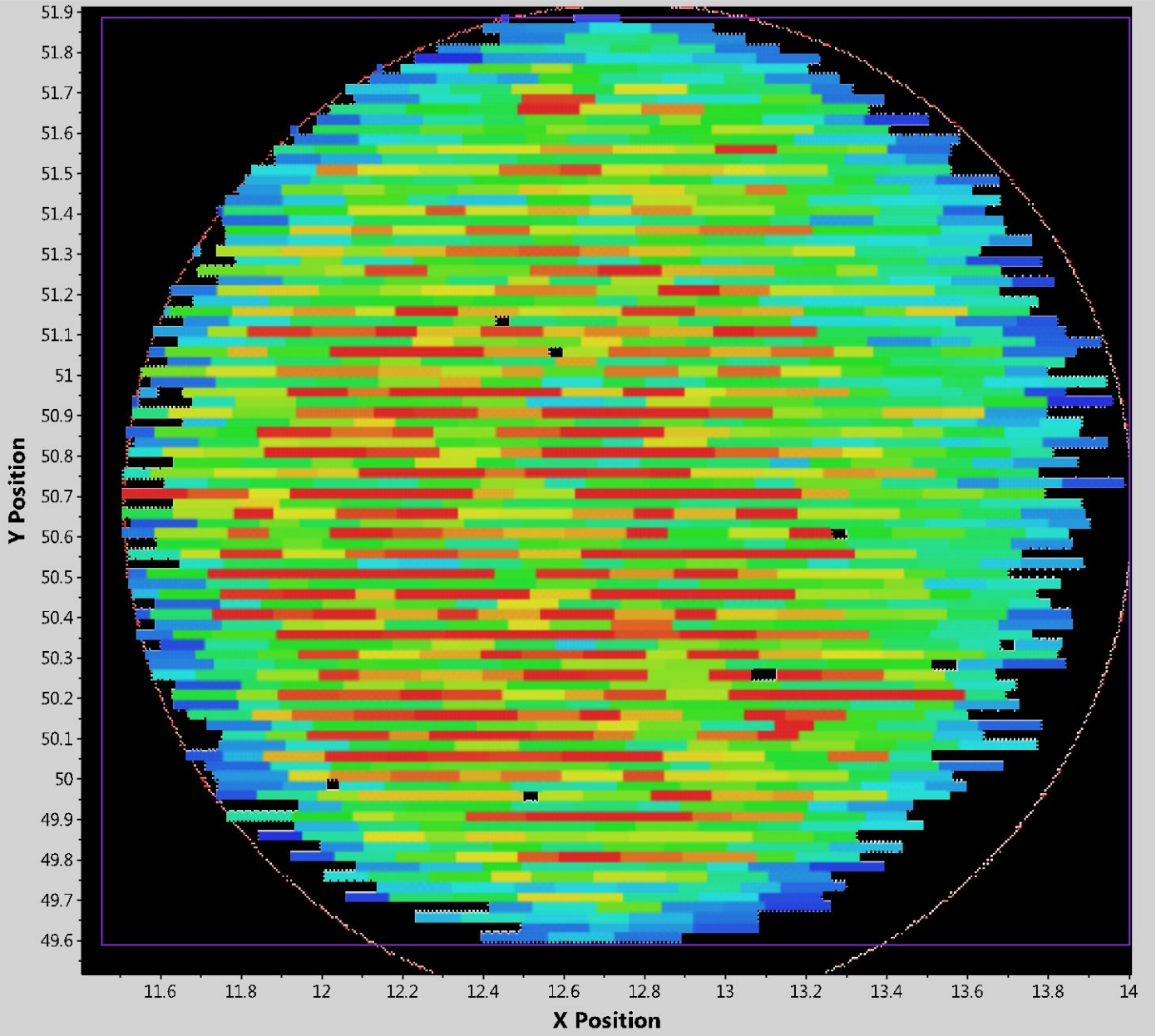
single spot
human serum 1:10 dilution
sinapinic acid matrix

Laser 1.7 μJ, Extraction 2.7 kV,

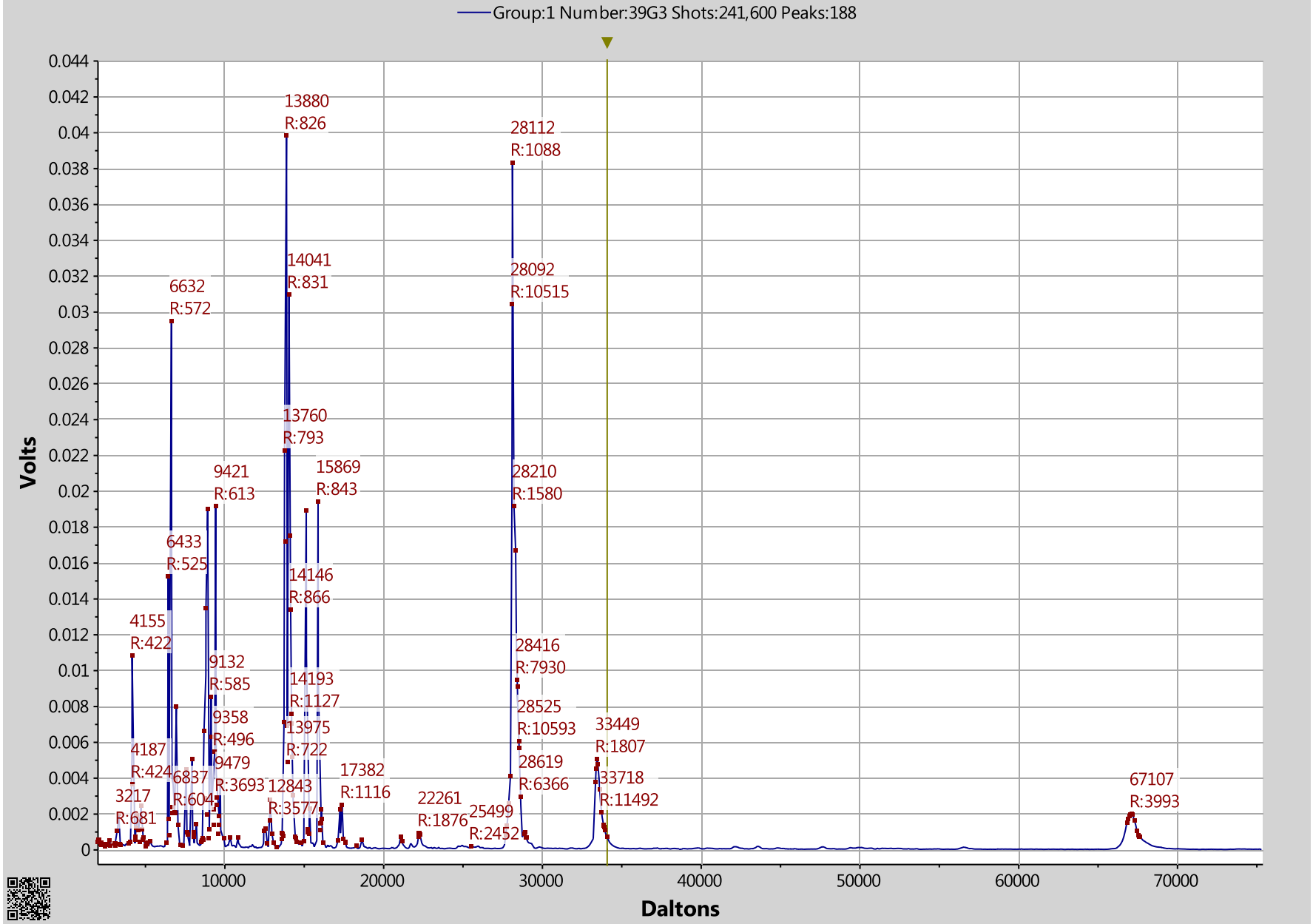
Delay 2050 ns, 1kHz

Raster 25 μm, 0.5 mm/s, 200 shots/sp

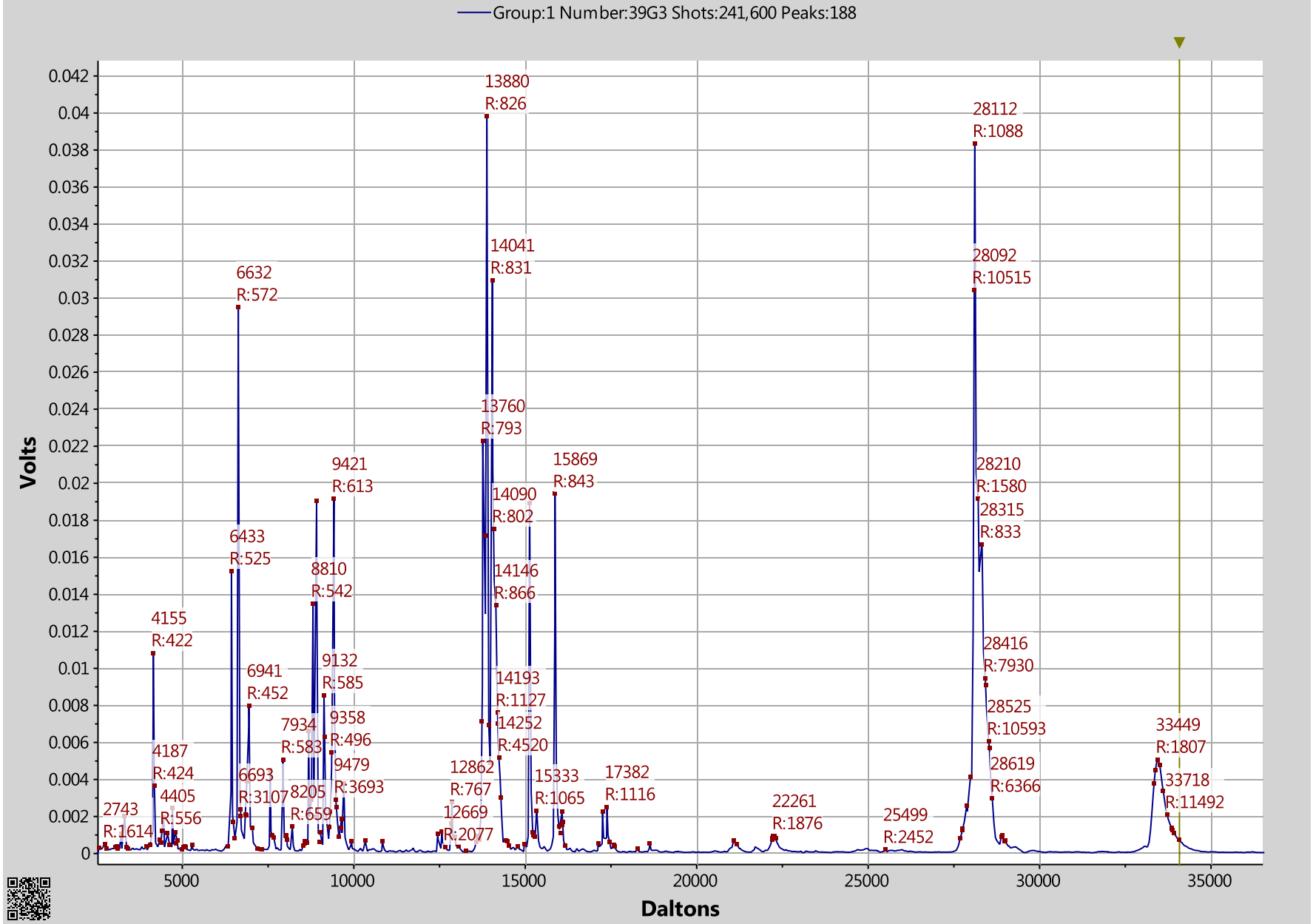
Image of single spot 25x100 µm pixels (241,600 laser shots)



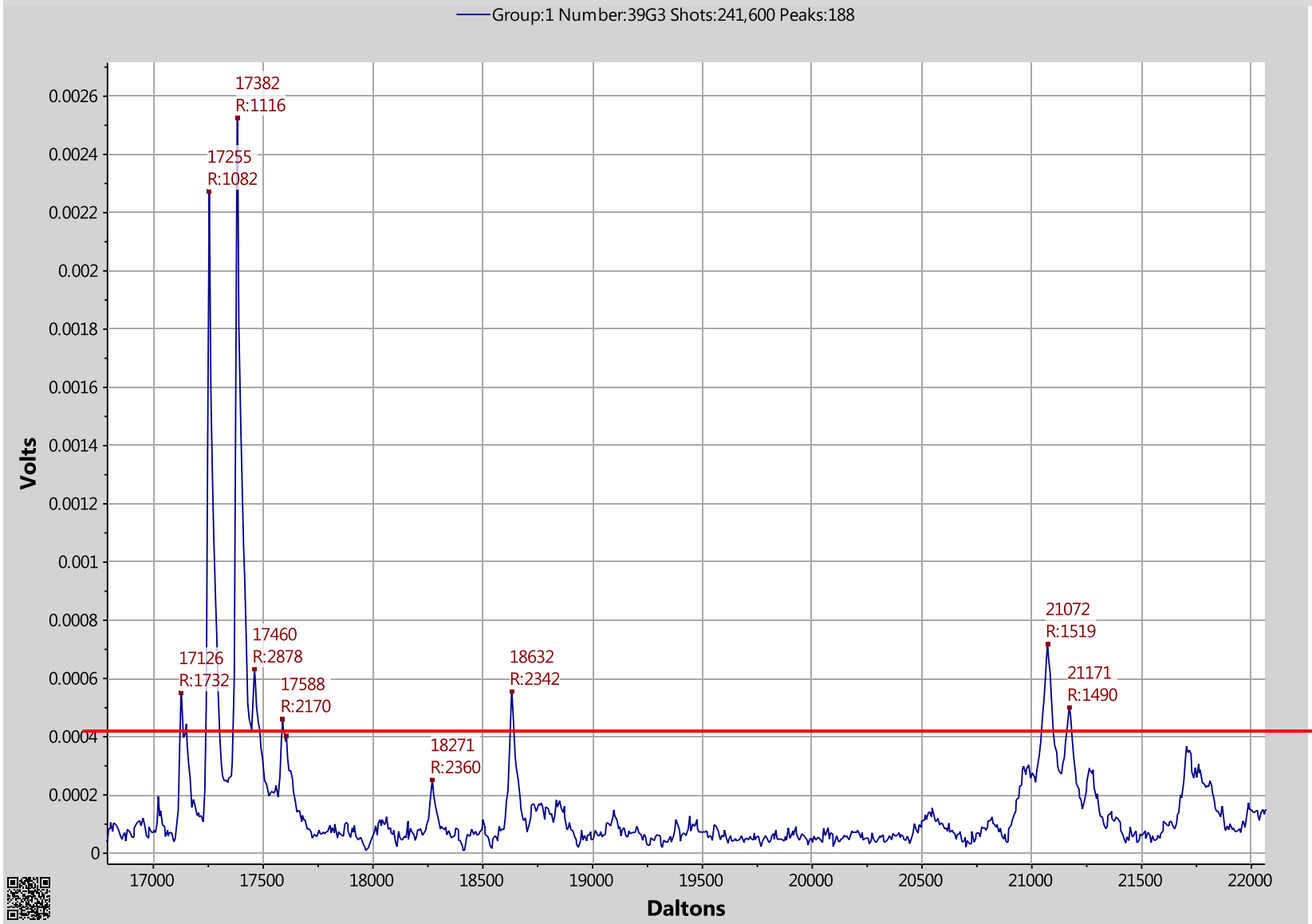
Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



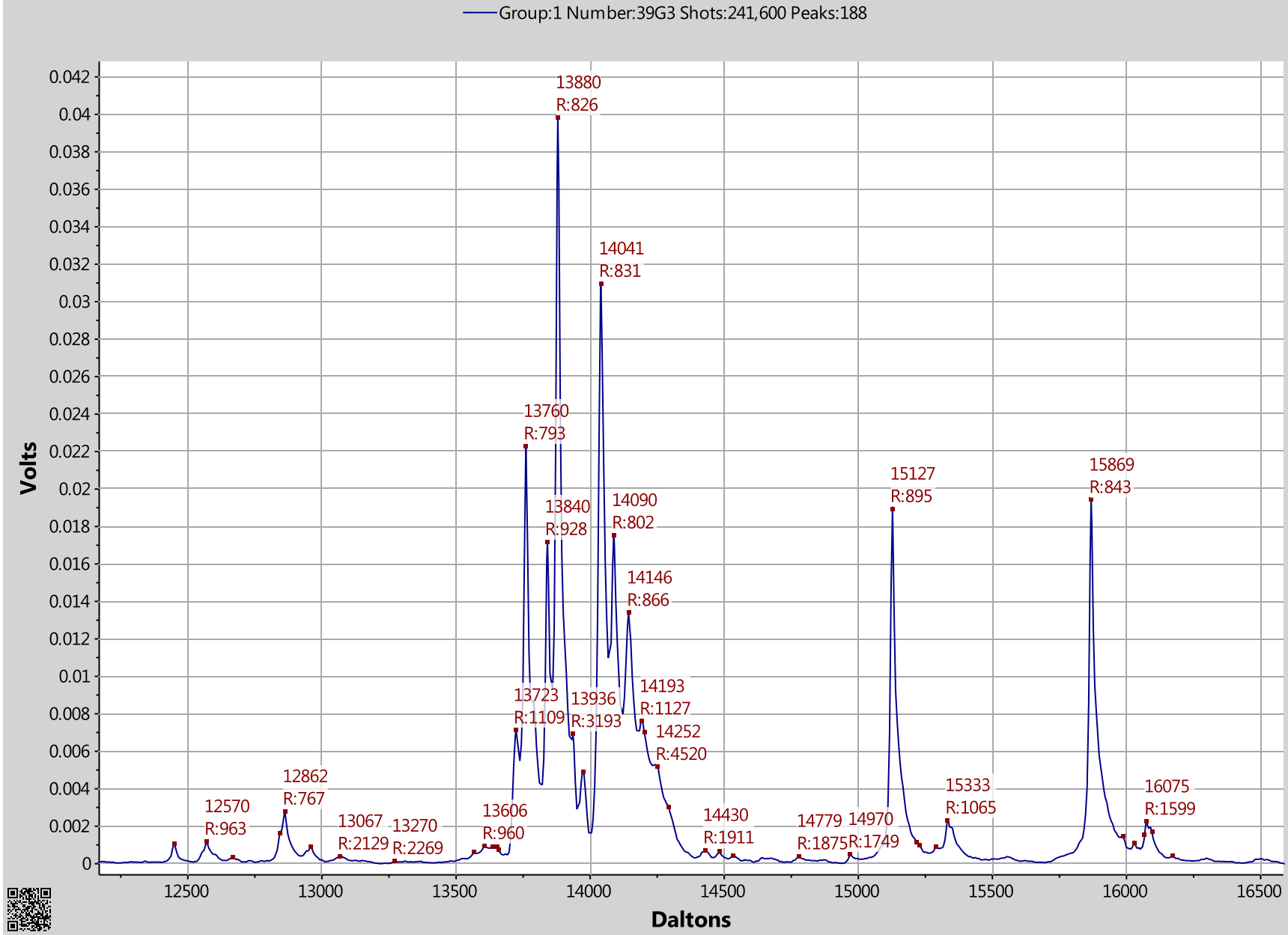
Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



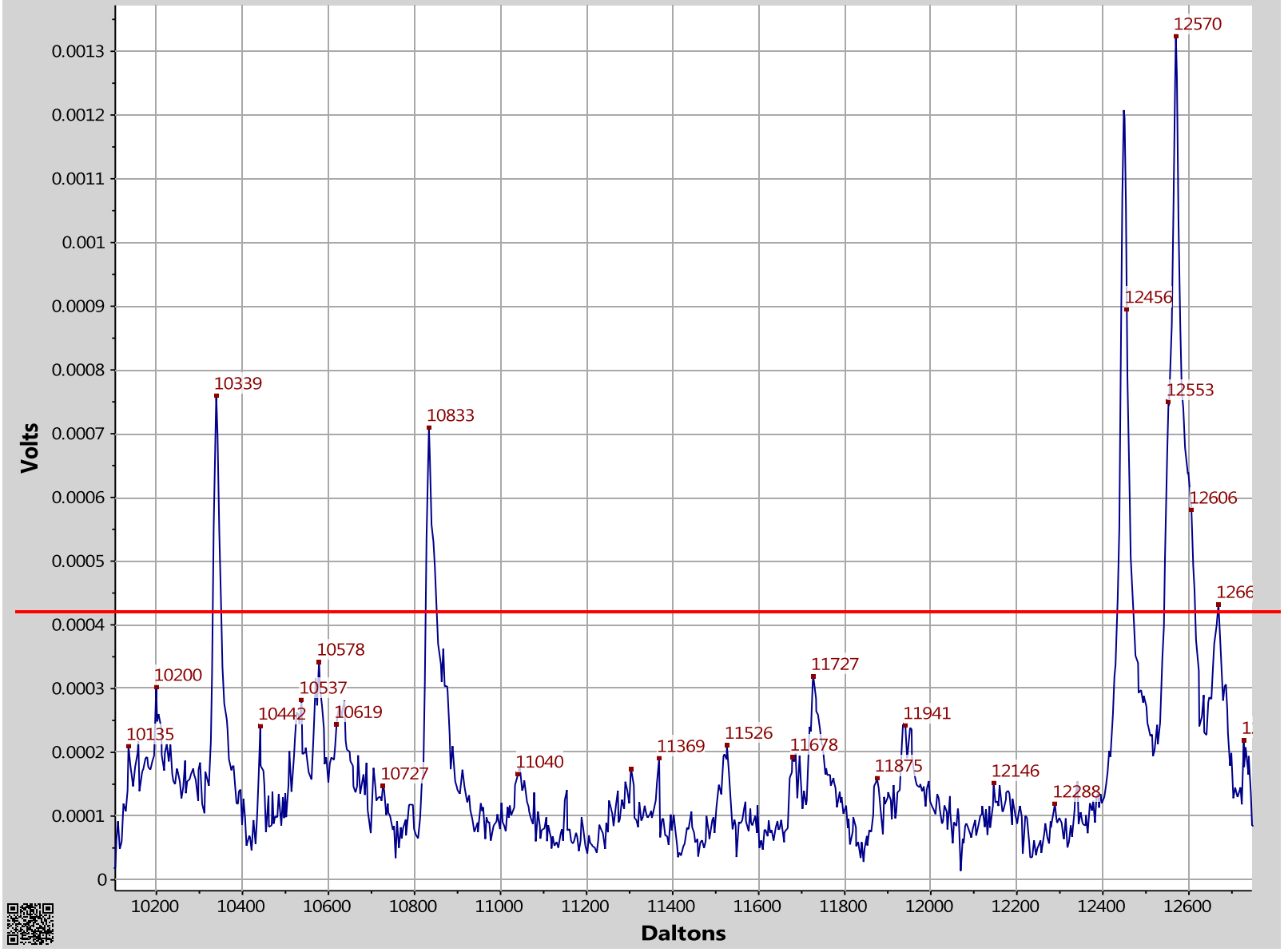
Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



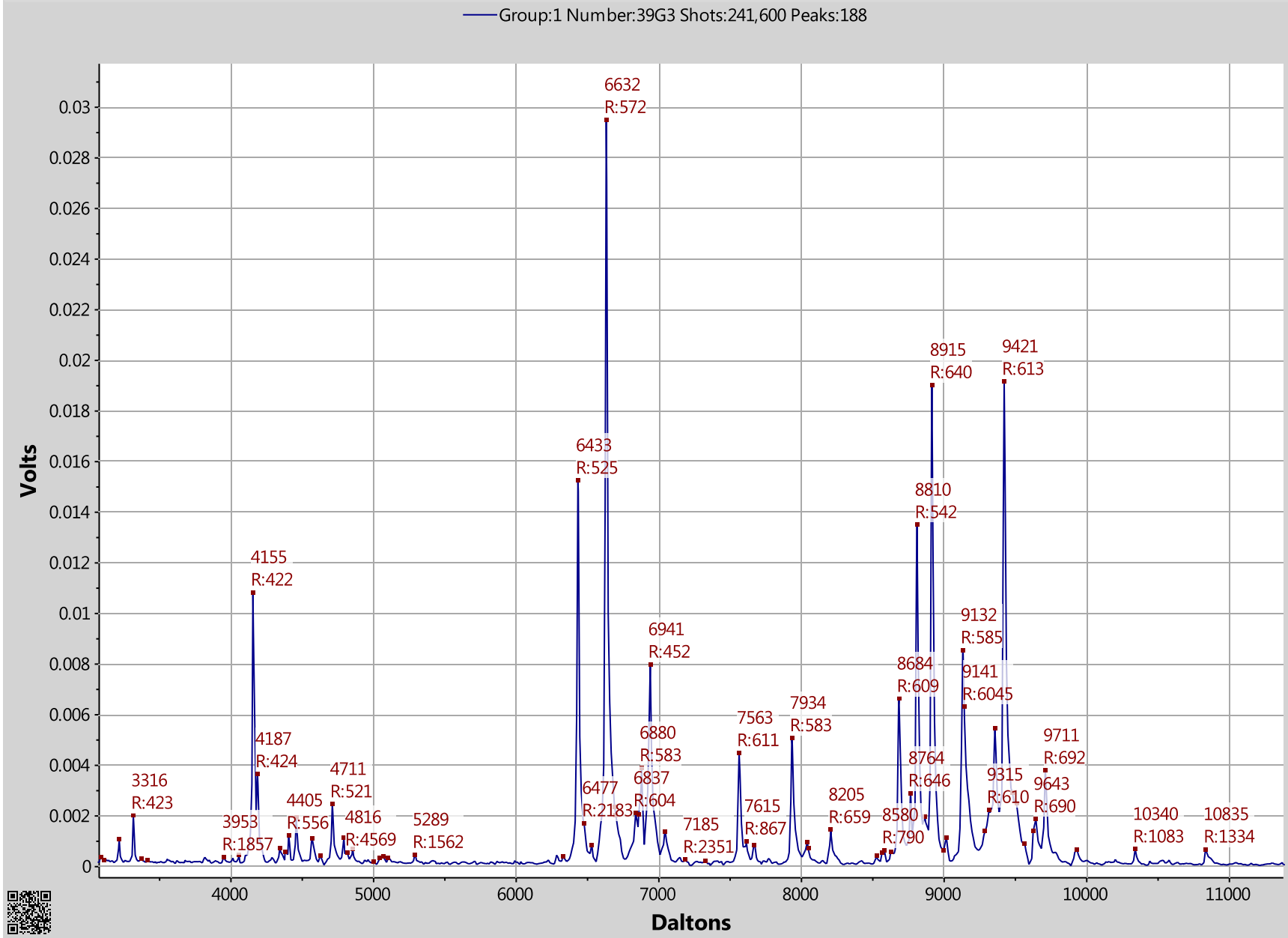
Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



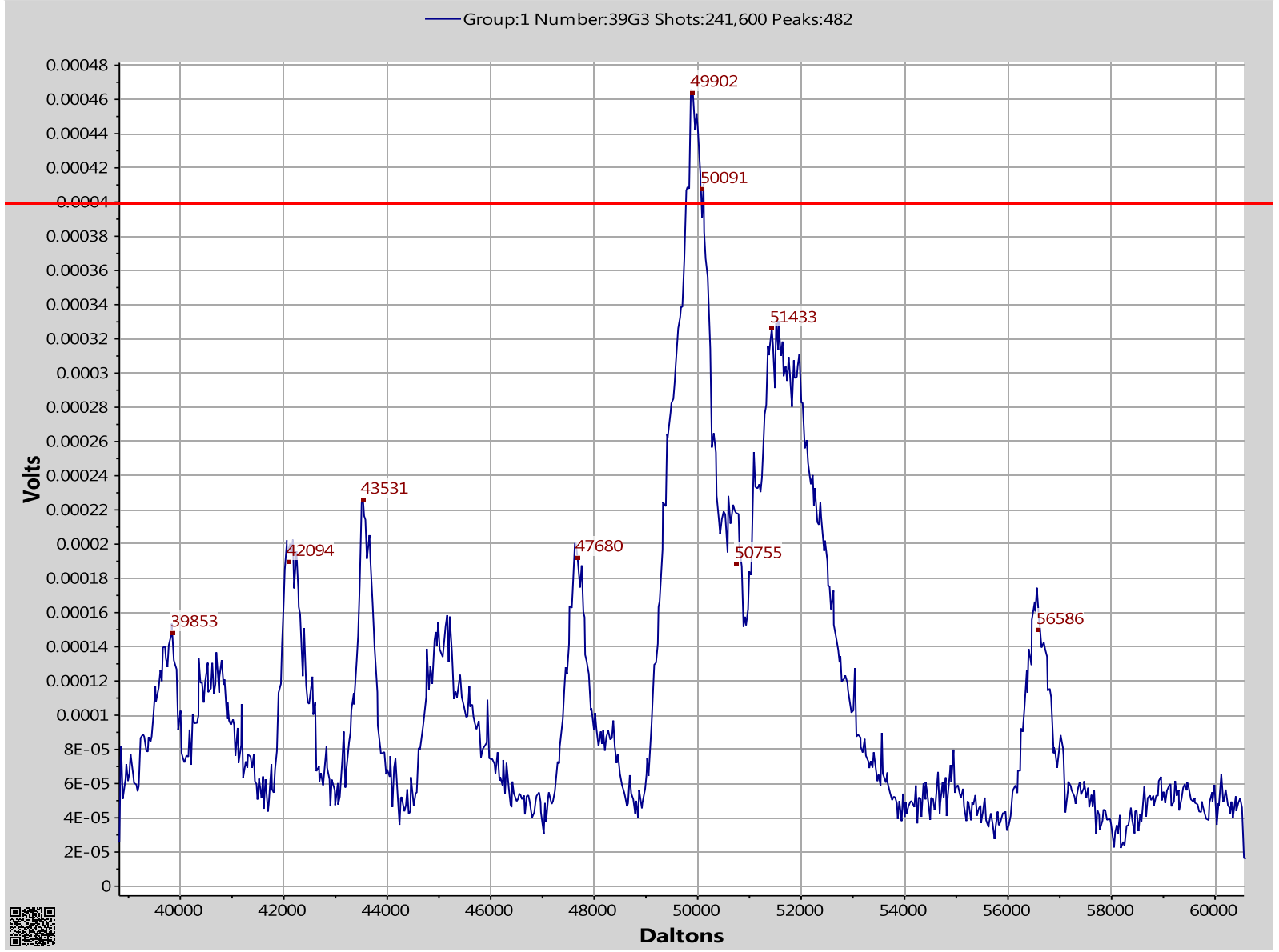
Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



Summed spectrum of single spot 25x100 µm pixels (241,600 laser shots)



1%

Examples of quantitation by MALDI-TOF

- Hemoglobin A1C

  Whole blood diluted 1:2000 in sinapinic acid

  Analysis and quantitation of glycated hemoglobin by matrix assisted laser desorption ionization time of flight mass spectrometry, S. J. Hattan et al, . J Am Soc Mass Spectrom. 2016 Jan 5. [Epub ahead of print] PubMed PMID: 26733405.

Hemoglobin from whole blood 1:2000 dilution in HCCA matrix

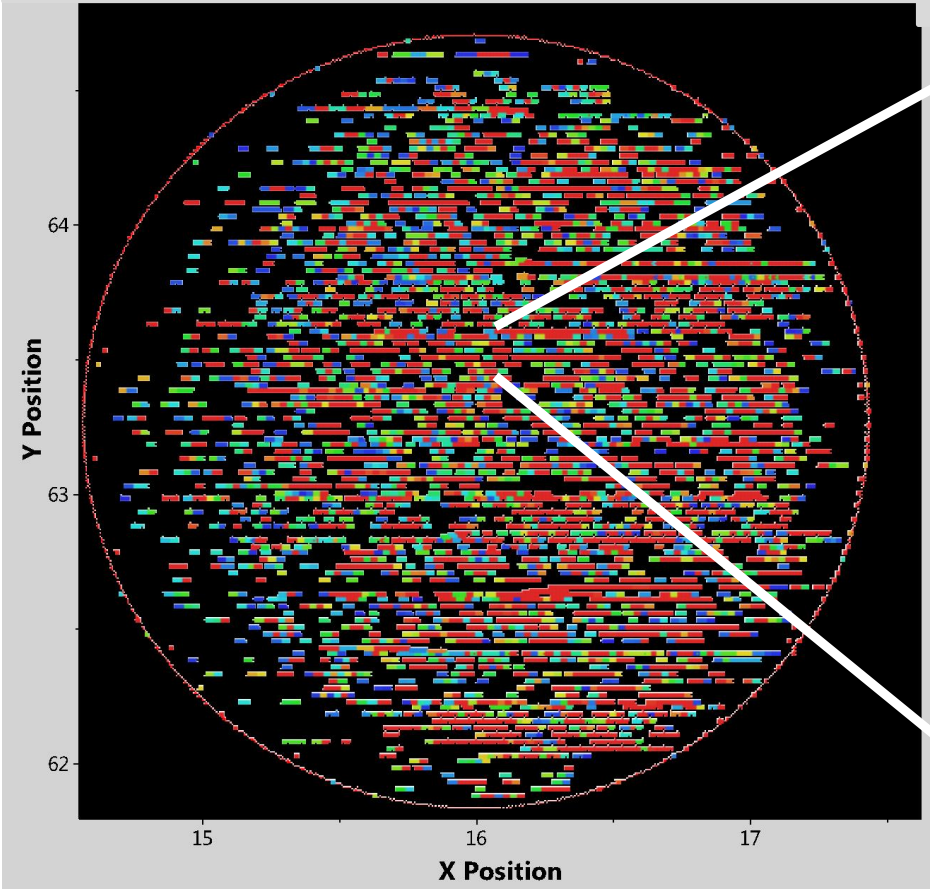
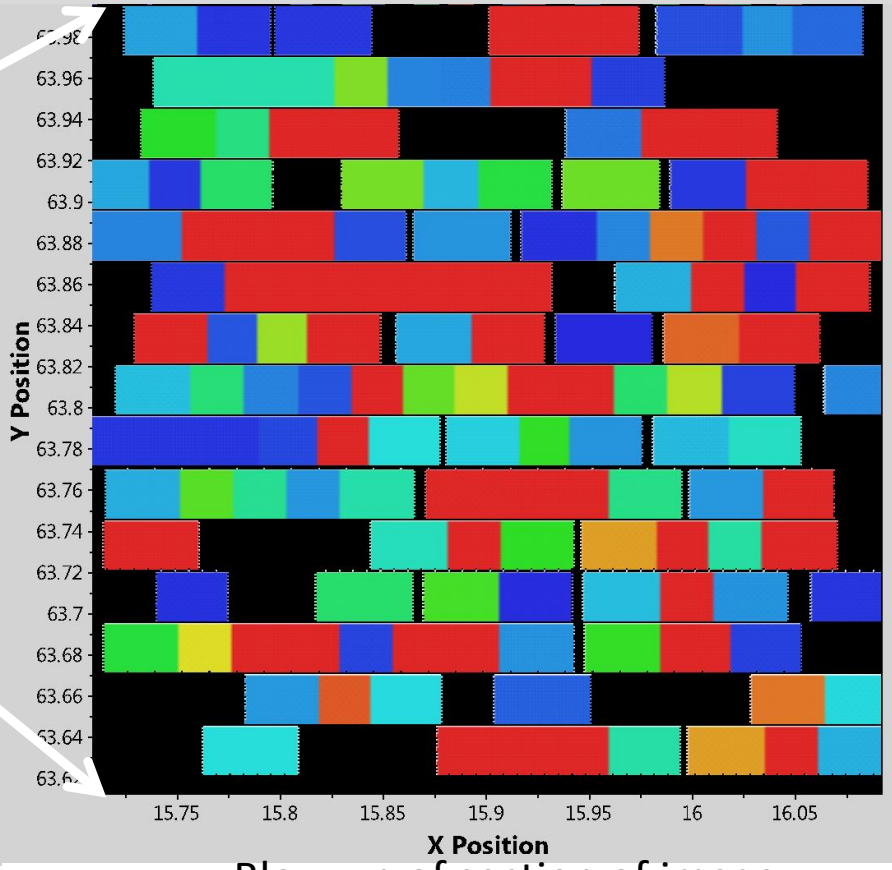
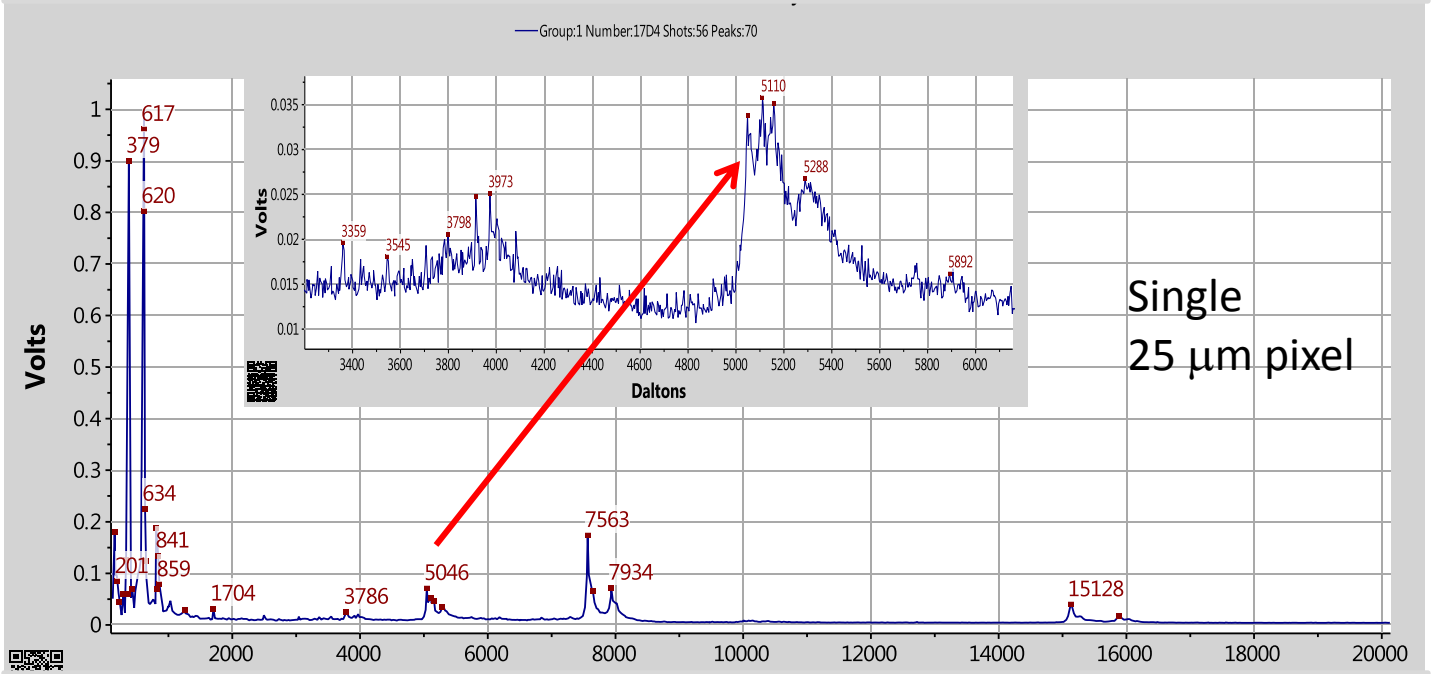


Image of 2.6 mm spot (450,000 laser shots) with 25 μm pixels,
HCCA matrix 25 μm raster @2 mm/s 7700 pixels @56 shots/pixel saved
5 kHz laser, 90 pixels/s, 90 s acquisition



Blow-up of section of image
Showing 25 μm pixels


Group:1 Number:17D4 Shots:56 Peaks:70
Volts
Daltons
617
379
620
634
841
201
859
1704
3786
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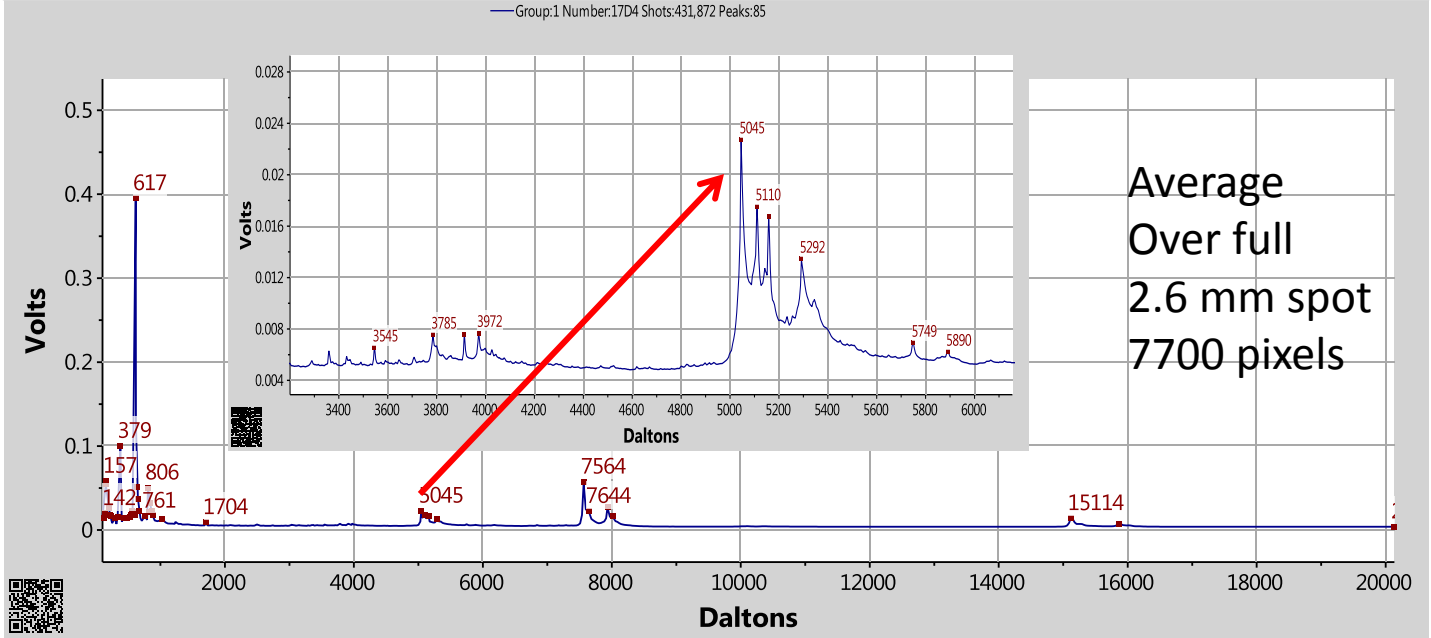
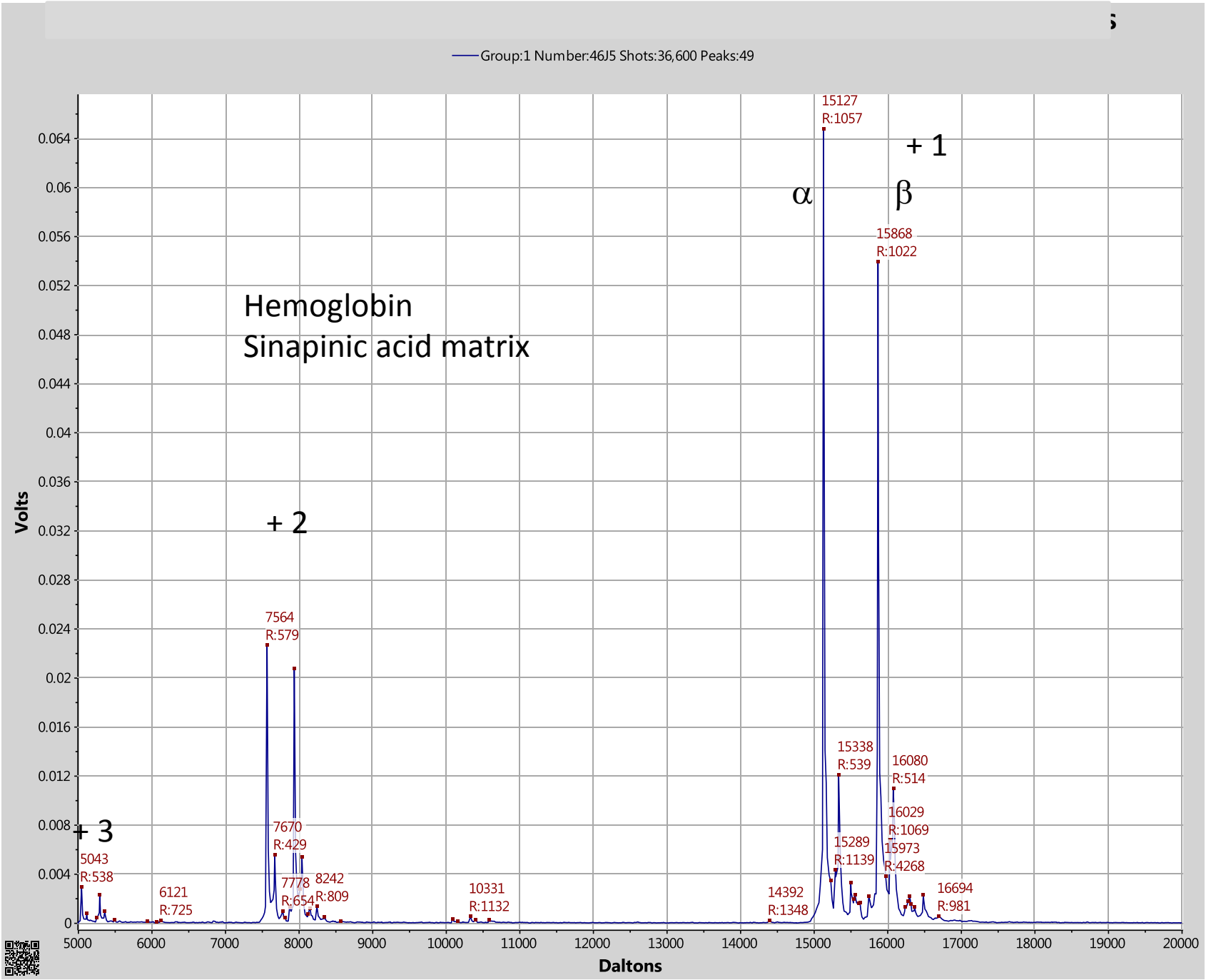
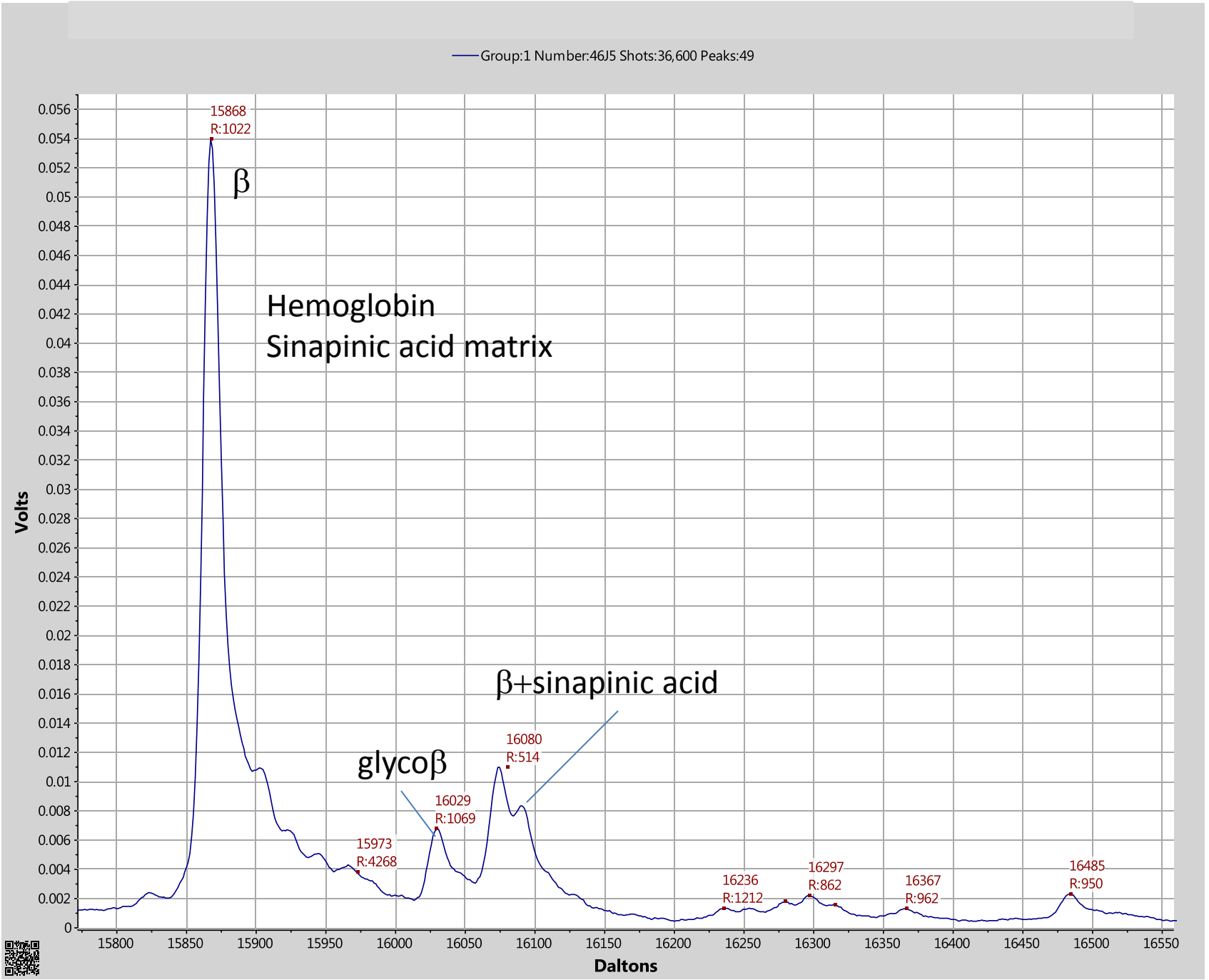
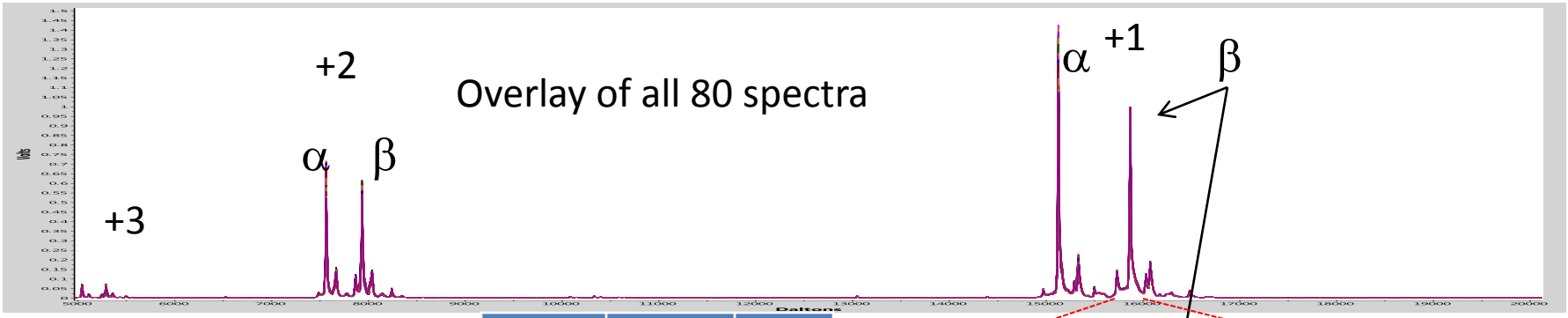
7563
7934
15128
3359
3545
3798
3973
5110
5288
5892
Single
25 µm pixel


Group:1 Number:17D4 Shots:431,872 Peaks:85
Volts
Daltons
617
379
157
806
142
761
1704
5045
7564
7644
15114
3545
3785
3972
5045
5110
5292
5749
5890
Average
Over full
2.6 mm spot
7700 pixels

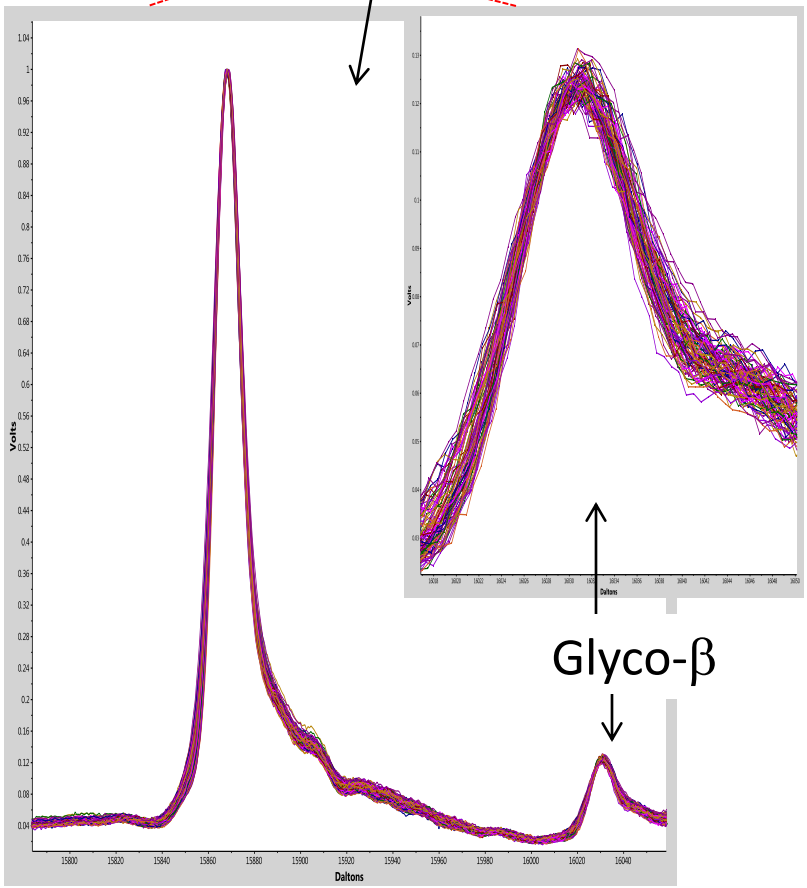

Group:1 Number:46J5 Shots:36,600 Peaks:49
Hemoglobin
Sinapinic acid matrix
+ 1
α
β
+ 2
+ 3
15127
R:1057
15868
R:1022
15338
R:539
16080
R:514
16029
R:1069
15973
R:4268
15289
R:1139
14392
R:1348
16694
R:981
10331
R:1132
7564
R:579
7670
R:429
7778
R:654
8242
R:809
6121
R:725
5043
R:538
Volts
Daltons
0
0.004
0.008
0.012
0.016
0.02
0.024
0.028
0.032
0.036
0.04
0.044
0.048
0.052
0.056
0.06
0.064
5000
6000
7000
8000
9000
10000
11000
12000
13000
14000
15000
16000
17000
18000
19000
20000


Group:1 Number:46J5 Shots:36,600 Peaks:49
15868
R:1022
β
Hemoglobin
Sinapinic acid matrix
β+sinapinic acid
glycoβ
16080
R:514
16029
R:1069
15973
R:4268
16236
R:1212
16297
R:862
16367
R:962
16485
R:950
Volts
Daltons
0.056
0.054
0.052
0.05
0.048
0.046
0.044
0.042
0.04
0.038
0.036
0.034
0.032
0.03
0.028
0.026
0.024
0.022
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
15800
15850
15900
15950
16000
16050
16100
16150
16200
16250
16300
16350
16400
16450
16500
16550



MALDI plate

	GlyHb/Hb%	CV %
A	13.52	1.39
B	13.48	0.71
C	13.20	0.62
D	13.07	0.99
E	13.07	0.96
F	13.11	0.90
G	12.98	0.67
H	12.99	1.19
I	13.05	0.96
J	12.99	0.99
K	13.08	0.92
L	13.01	0.66
M	13.16	1.00
N	13.15	0.84
O	13.22	0.70
P	13.17	0.85
Average	13.14	0.90
Std Dev	0.16	
Rel Std Dev	1.22	



Conclusion

MALDI-TOF MS analysis and quantitation of glycated-βHb is both feasible and practical and contains some distinct advantages over currently practiced methods. The approach is accurate, precise, sensitive, rapid and requires minimum sample workup. The analysis is calibrated with NGSP validated, commercially available, reference materials and is demonstrated to be portable between different laboratories and different mass spectrometers. Both quantification and mass calibration are performed by using signals internal to each sample, thereby eliminating the need for addition of external reference materials. Additional and independent measurements are made simultaneously and these measurements serve to strengthen the confidence of the primary % glycated-βHb measurement and potentially aid in Hb "variant-form" determination.