Portable MALDI-TOF Mass Spectrometer for Bioaerosol Detection

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The 14th Harsh-Environment Mass Spectrometry Workshop,
September 26-29, 2022

Biothreat detection by mass spectrometry

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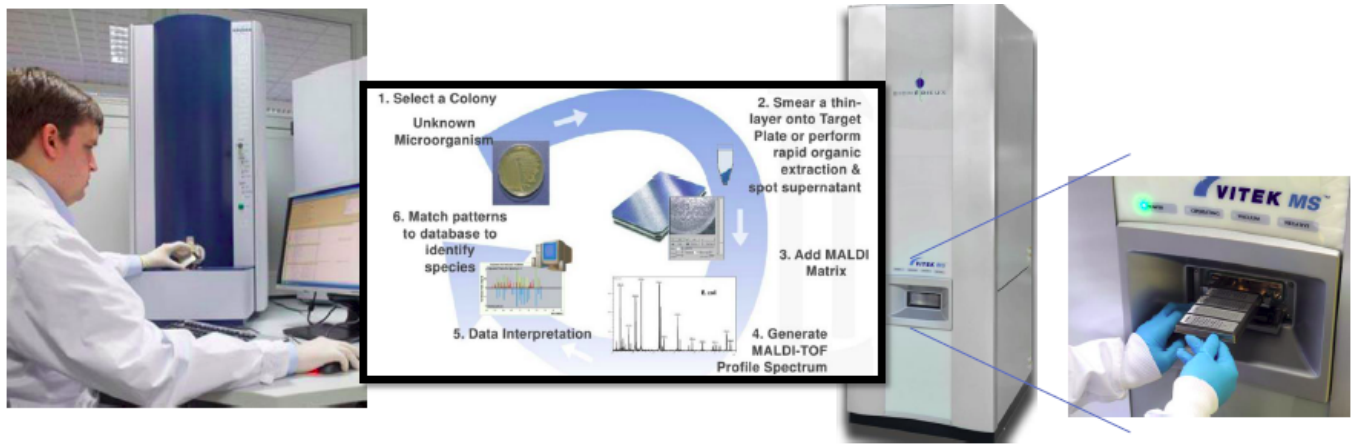
MALDI-TOF is widely used in clinical diagnostics:



Bruker/BD Biotyper

- Over 2000 units sold
- Sales price ~$350K
- >6900 strains including *Mycobacteria tuberculosis*
- Strain level information
- 100's of millions samples run
- FDA and CE-IVD cleared

Shimadzu/bioMérieux Vitek MS

- Larger sales volumes worldwide
- Sales price ~$350K
- >15,000 strains of >1000 species in database
- Bacteria, mycobacteria, nocardia, moulds and yeast
- Strain and antibiotic susceptibility info
- FDA and CE-IVD cleared

- Both commercial systems require skilled user for manual sample collection and culturing (12-24 hrs.)
- Zeteo Tech has developed a fully automated system providing results in about 5 min, which was later commercialized by spin-off company BioFlyte (BioTOF system).

BioFlyte BioTOF

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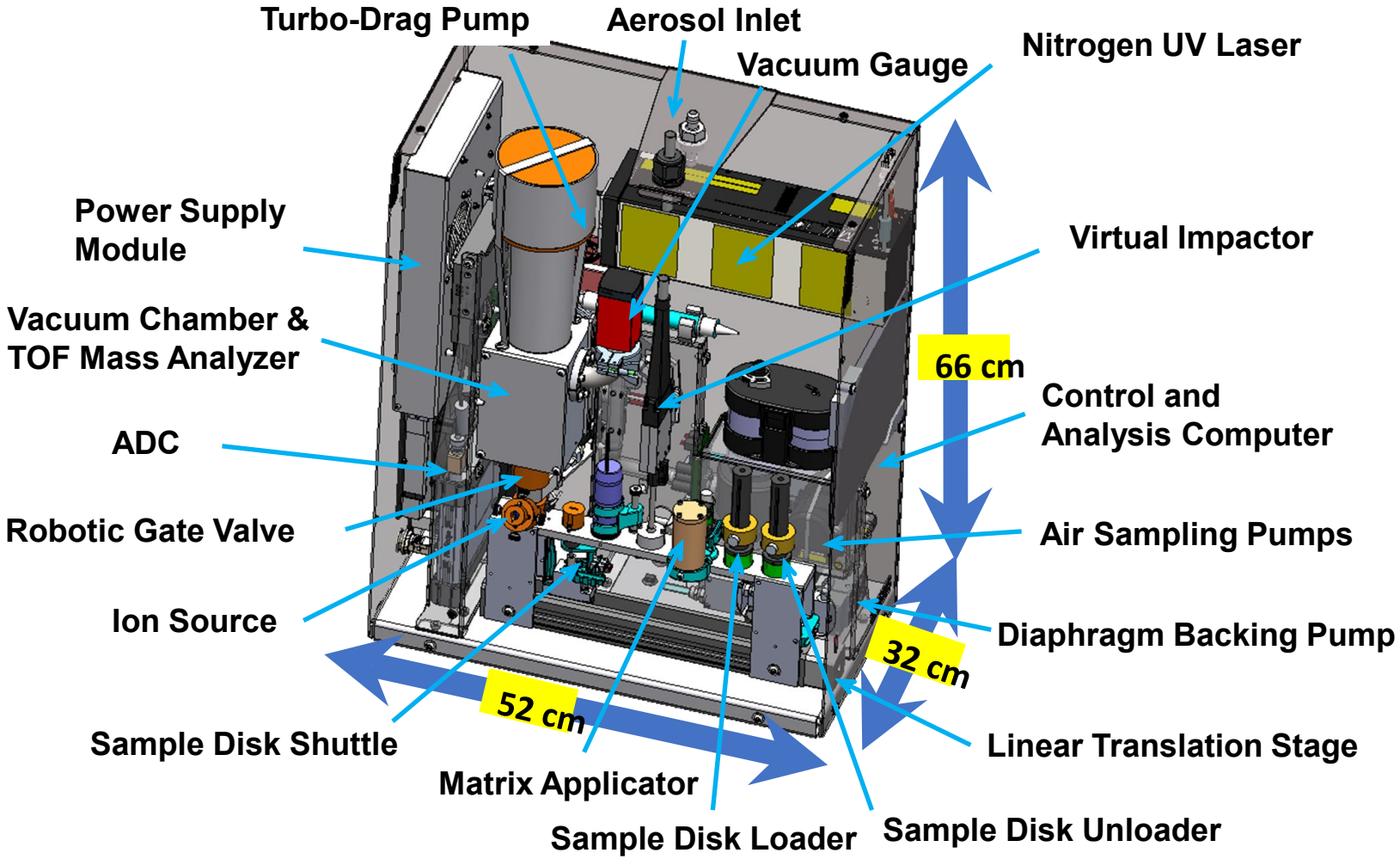

Weight
~25 kg
Power
50 W standby, 65 W measuring, 100 W pumping down

Automated BioTOF™ system rapidly identifies single biological threats with high specificity and sensitivity in a modestly cluttered environment. To extend this capability to more complex environments modification of the mass analyzer is required to increase mass resolution enabling accurate deconvolution of overlapping peaks.

SIMION model of the TOF mass analyzer

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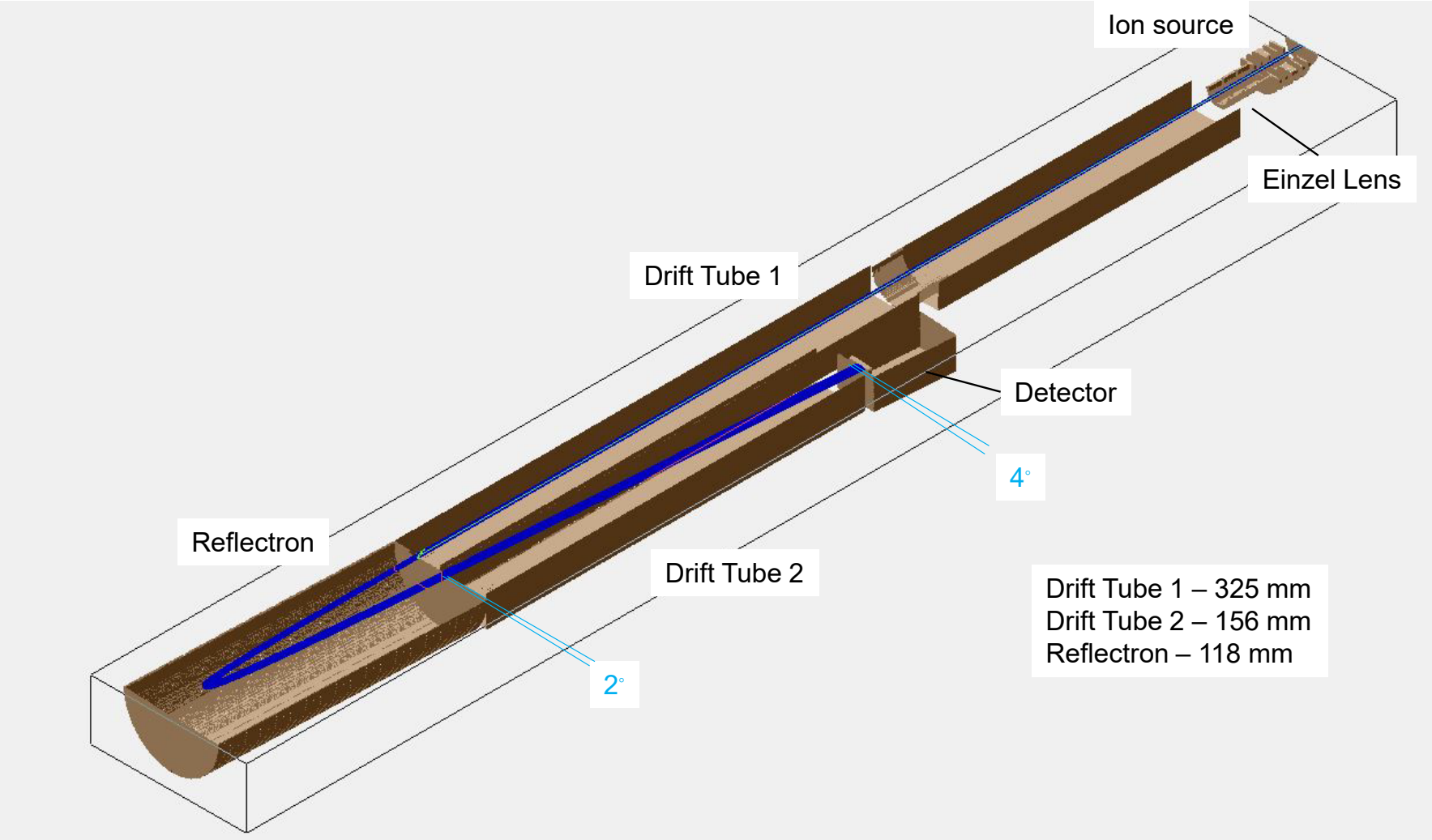

Two-stage acceleration and a single-stage reflectron configuration of TOF mass analyzer was chosen. The specific dimensions were determined to provide first-order space and velocity focusing.

Delayed extraction

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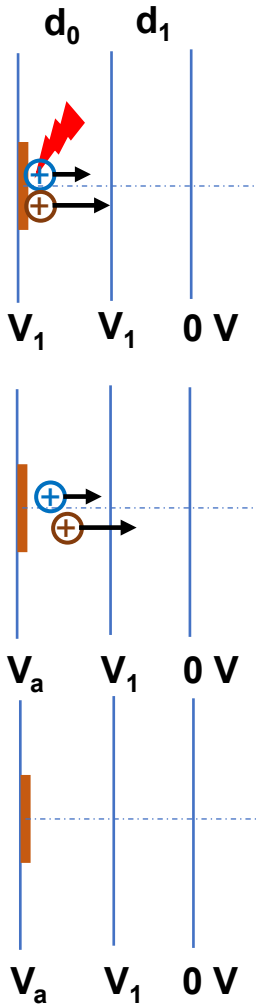
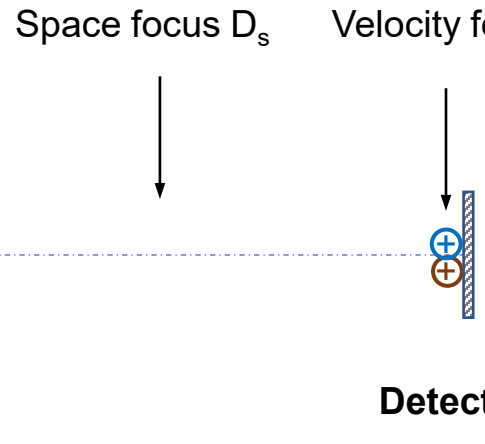


Brown ion has higher initial velocity

Blue ion will acquire more energy in electric field

Ions arrive at focus plane simultaneously



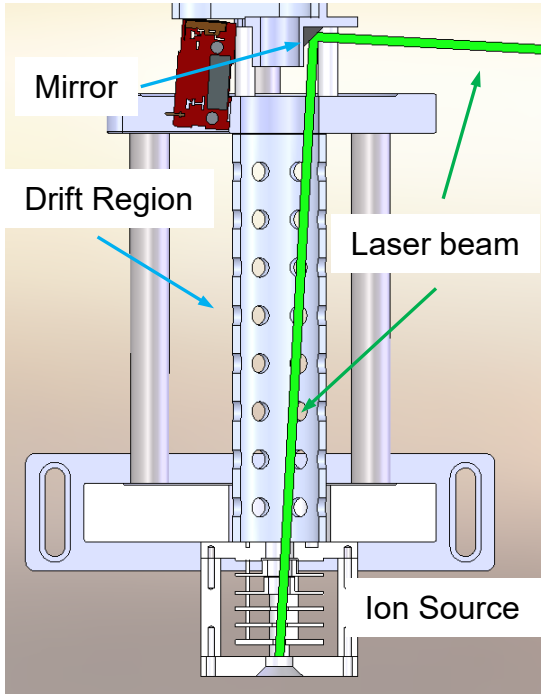
$$D_s = 2d_0y^{3/2}[1-(d_1/d_0)/(y^{1/2}+y)]$$

$$D_v = D_s = (2d_0y)^2/(v_n\Delta t)$$

where $y=V_a/(V_a-V_1)$, $v_n = C\,(V_a/m)^{1/2}$, Δt is the delay time

With proper choice of parameters (pulse voltage, delay time) both ions arrive at the detector at the same time.

UV laser beam focusing



Thin lens equations:

$$\frac{1}{s'} = \frac{1}{s + \frac{(M^2\pi\omega_0^2)^2}{\lambda^2(s+f)}} + \frac{1}{f}$$

and

$$\omega = \frac{\omega_0 f}{\sqrt{(s-f)^2 + (\frac{\pi\omega_0^2}{\lambda})^2}}$$

For the 349 nm laser (model Explorer One 349-120) used in the system, the laser beam waist measured at the exit $\omega = 0.16$ mm and the divergence $2\theta = 3.0$ mrad, $M^2 = 1.3$ and $\omega_0 = M^2 \cdot \lambda/\pi\theta \approx 48$ μm.

For a fused silica lens with nominal focal length f = 200 mm (f = 193 mm at λ=349 nm), placed at distance 460 mm from laser exit window one can calculate s′= 310 mm and d ≈ 100 μm

Enhanced resolution TOF mass analyzer

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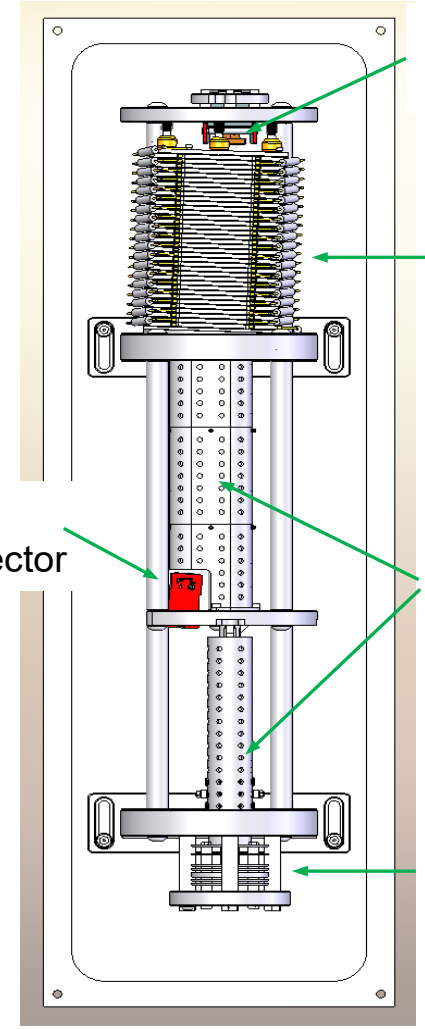
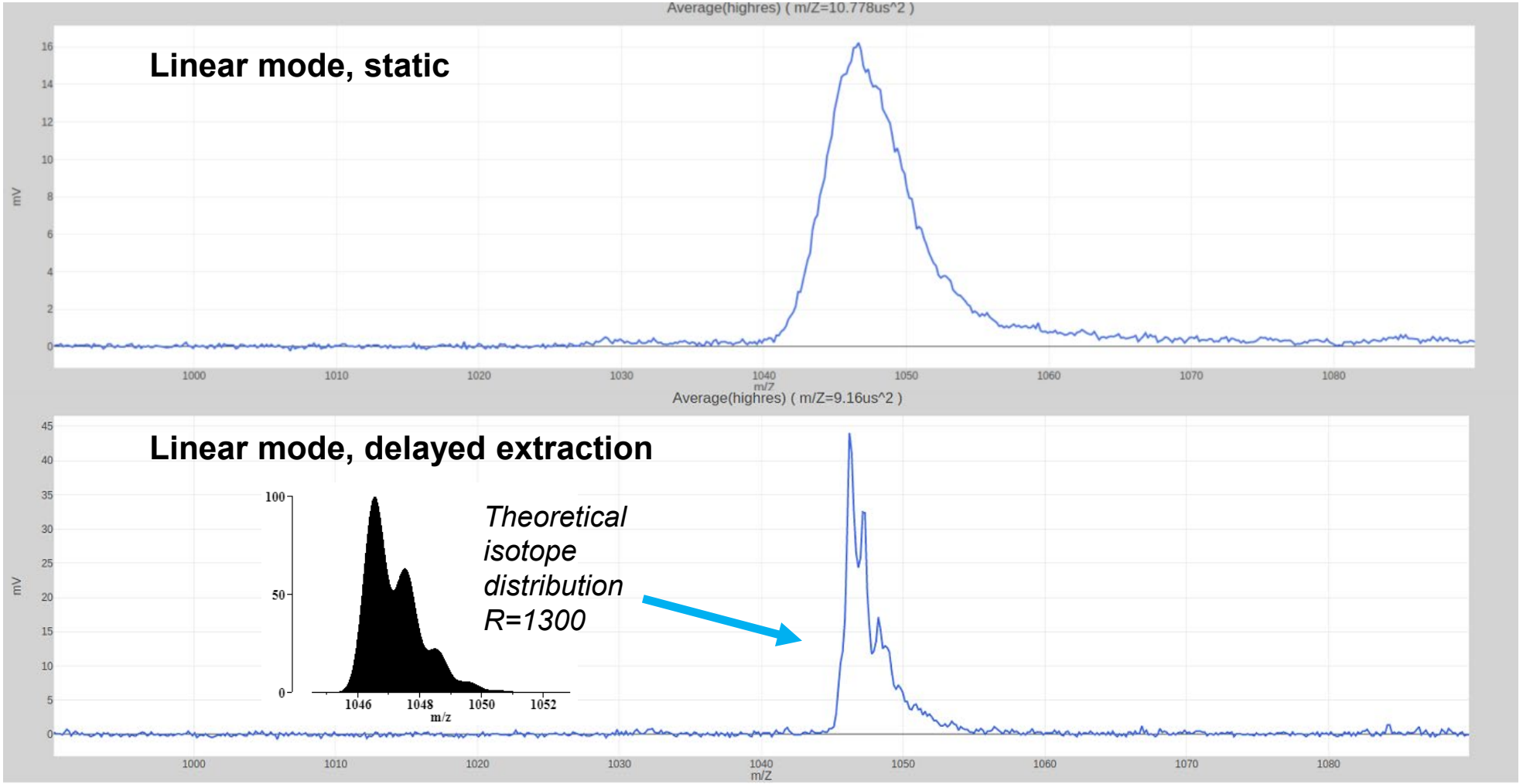


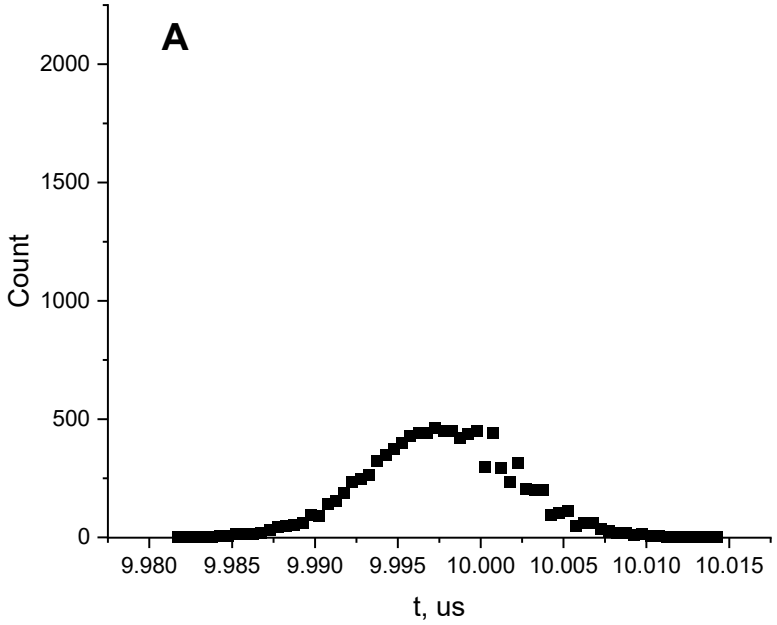
Based on simulation results TOF mass analyzer was designed and built. The overall length of mass analyzer is about 0.5 m.

Mass spectra of Angiotensin II, 10 pmole

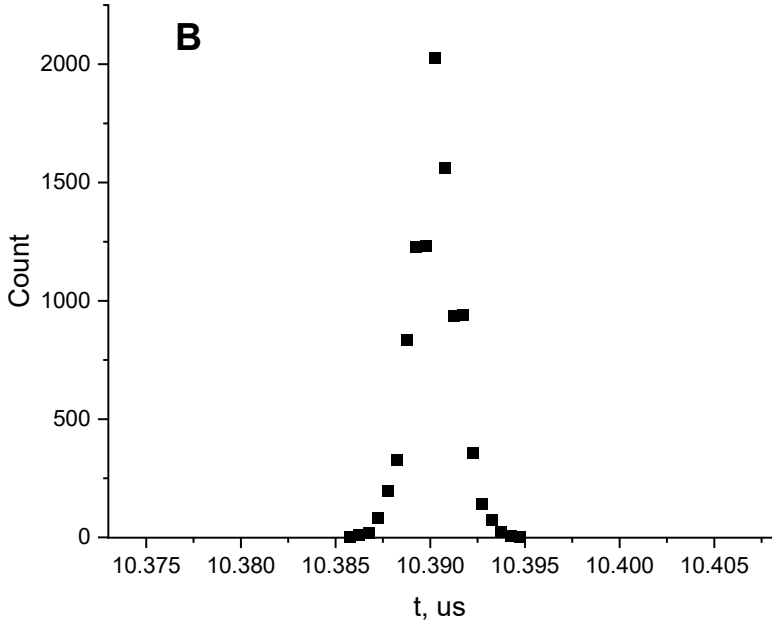


Delayed extraction allows to substantially improve mass resolving power in the same size TOF mass analyzer.

Linear Mode, SIMION modeling



Static **R=491**

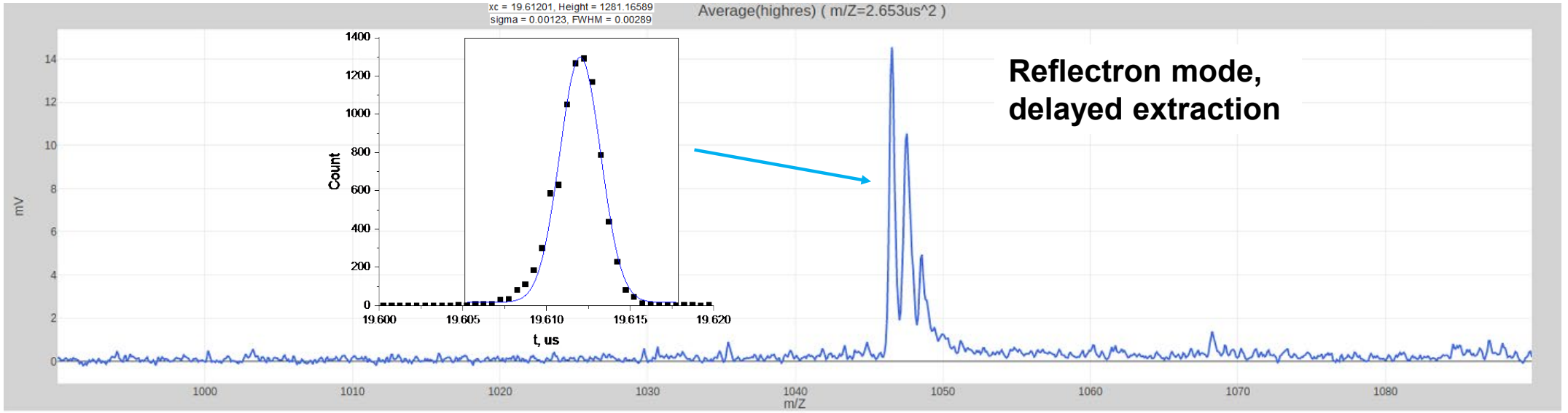


Delayed extraction, 400 ns **R=1910**

m/z = 1046, N=10000;
σ_x, σ_y = 100 μm; σ_z = 10 μm
σv_x, σv_y = 30 m/s;
v_z = 300 m/s, σv_z = 150 m/s

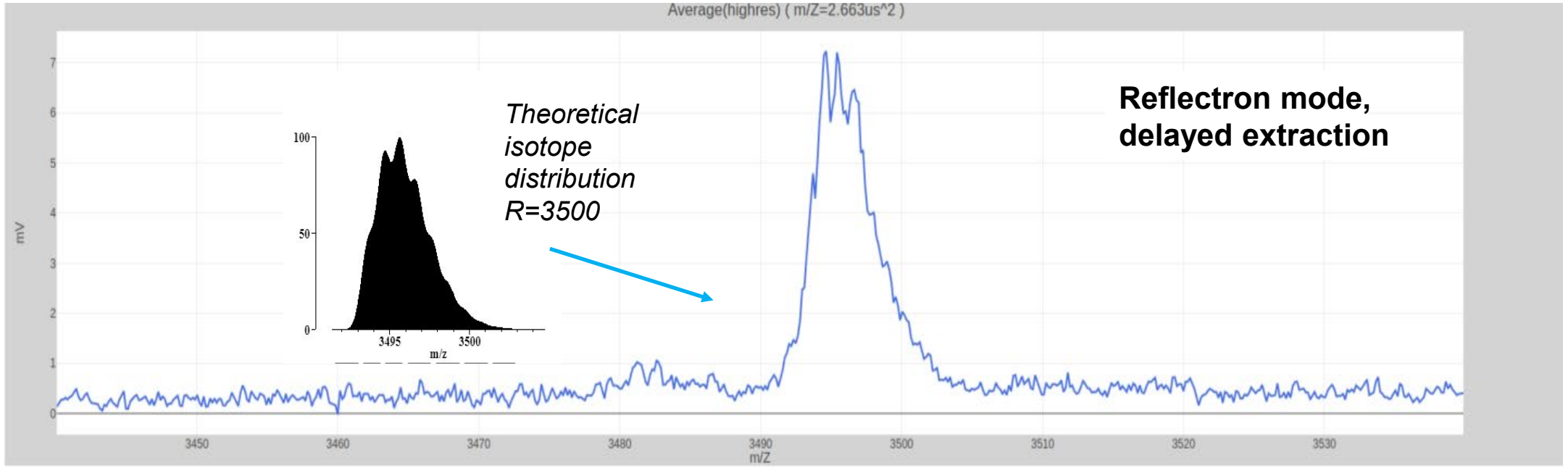
- Numeric modeling of *m/z*=1046 peak is in good agreement with experimental data
- The difference between theoretical and experimental peal width is explained by additional factors such as scattering on the grids, detector response time, HV switch jitter, etc. which results in additional peak broadening in comparison with the ideal model case
- Developed models allows further design optimization without “cutting metal”

Mass spectrum of Angiotensin II, 10 pmole acquired in reflectron mode



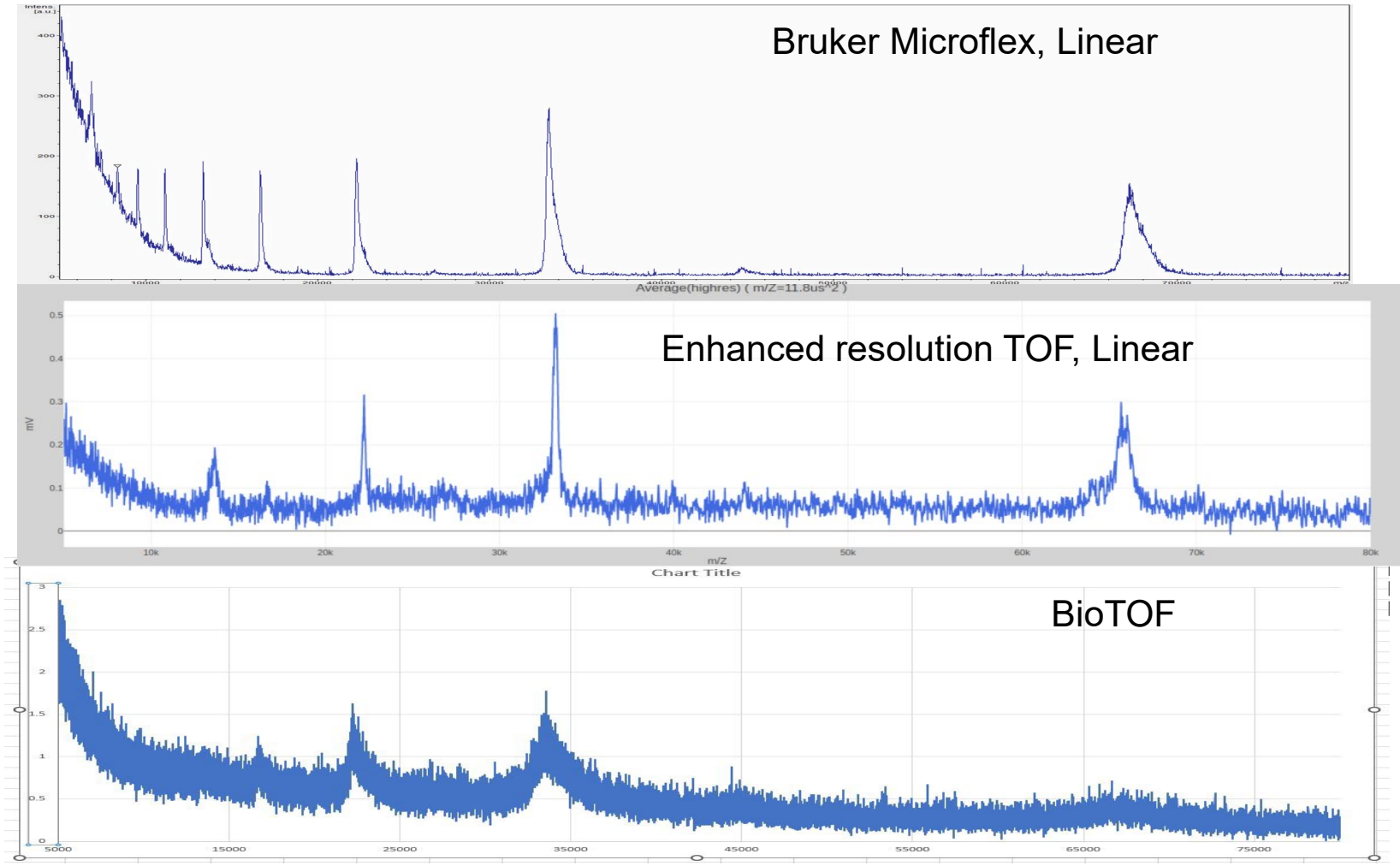
- Further improvement in mass resolving power (R=2600) was demonstrated when using the instrument in reflectron mode with delayed extraction
- Good agreement with SIMION modeling is also observed in this mode of operation when taking into account real system effects which broaden the peaks

Mass spectrum of Insulin chain B, 18 pmole acquired in reflectron mode



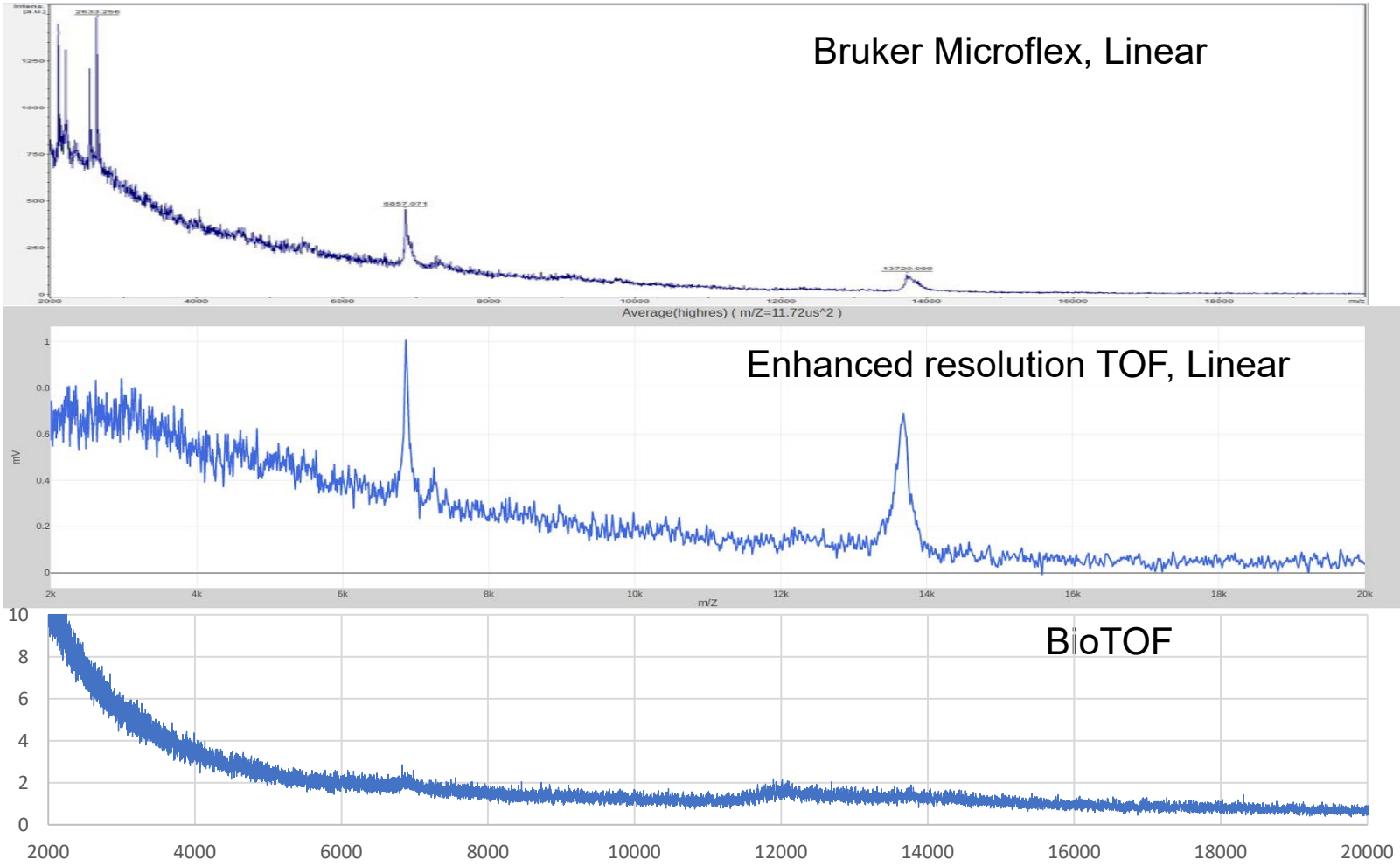
- Even better mass resolving power was demonstrated when analyzing higher mass peptides such as Insulin chain B (*m/z*=3494). Comparison with theoretical isotope distribution depicted in the inset gives an estimate of mass resolving power of about 3500.
- This higher resolving power is due to the fact that for first-order focusing of the initial ion distribution achieved by combination of delayed extraction and reflectron, the peak width (Δt) changes slowly with mass, while the ion time-of-flight (t) is directly proportional to $(m/z)^{1/2}$.

Mass spectra of BSA, 30 pmole acquired on different systems



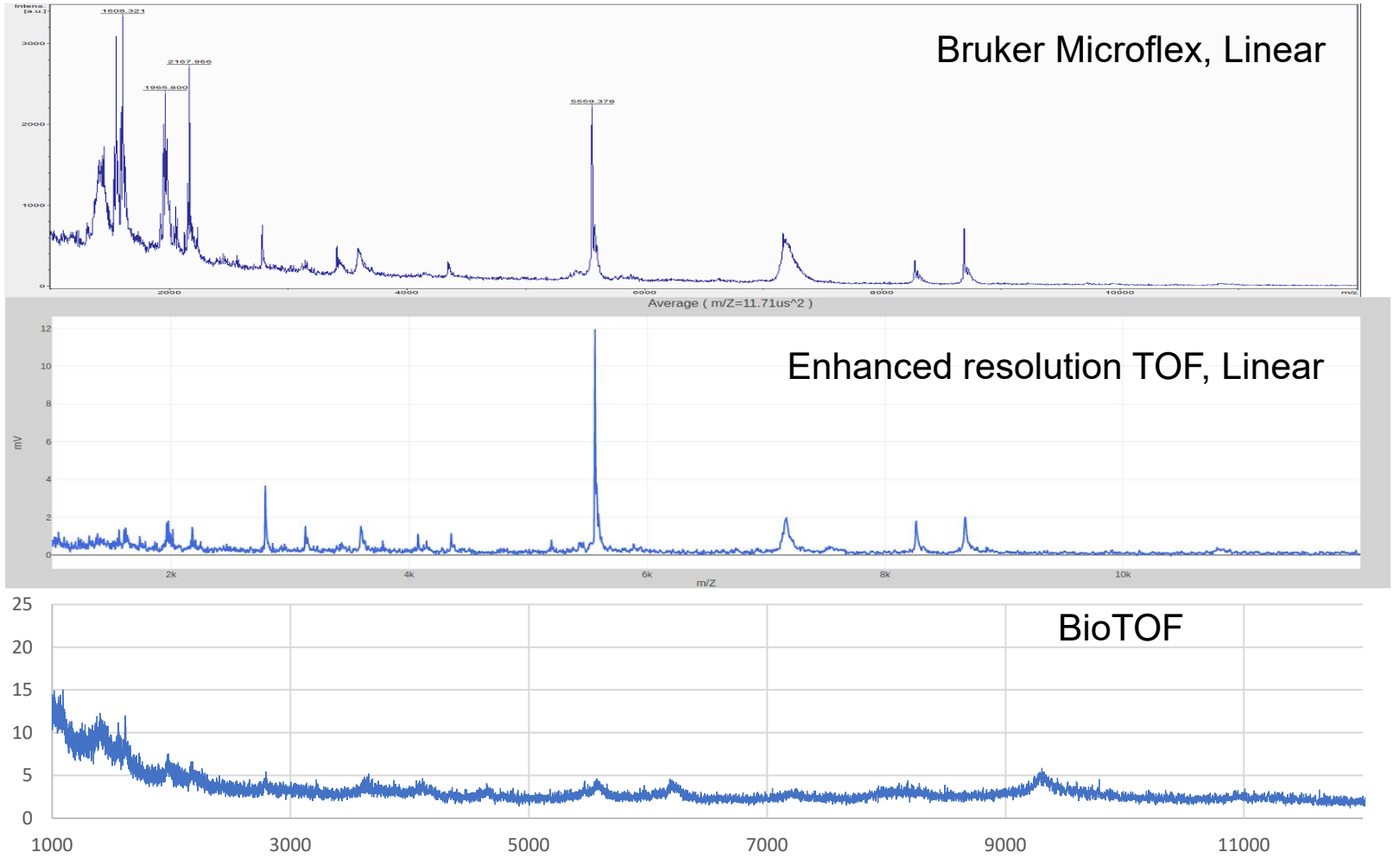
- Enhanced resolution TOF demonstrates significant improvement in comparison with BioTOF
- Mass resolving power on the enhanced resolution TOF is comparable to Microflex. Signal to noise ratio is slightly worse on this instrument because it utilizes smaller acceleration voltage (14 kV vs 19.5 kV).

Mass spectra of MS2 virus acquired on different systems



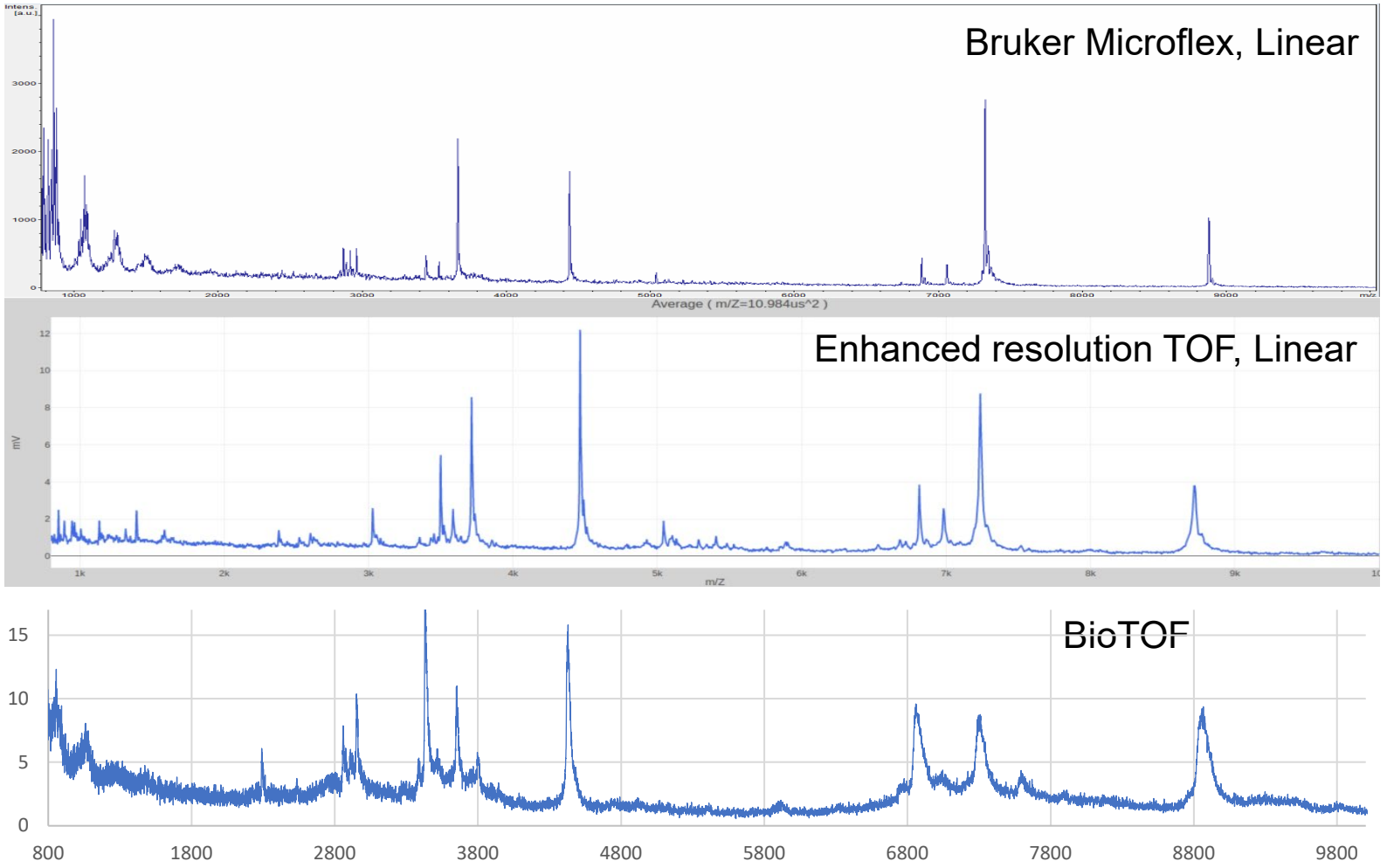
- The spectrum recorded on enhanced resolution TOF is comparable to the spectrum recorded on Microflex and significantly better when the spectrum acquired on BioTOF, which barely shows coat protein peaks

Mass spectra of *E. coli* K12 acquired on different systems



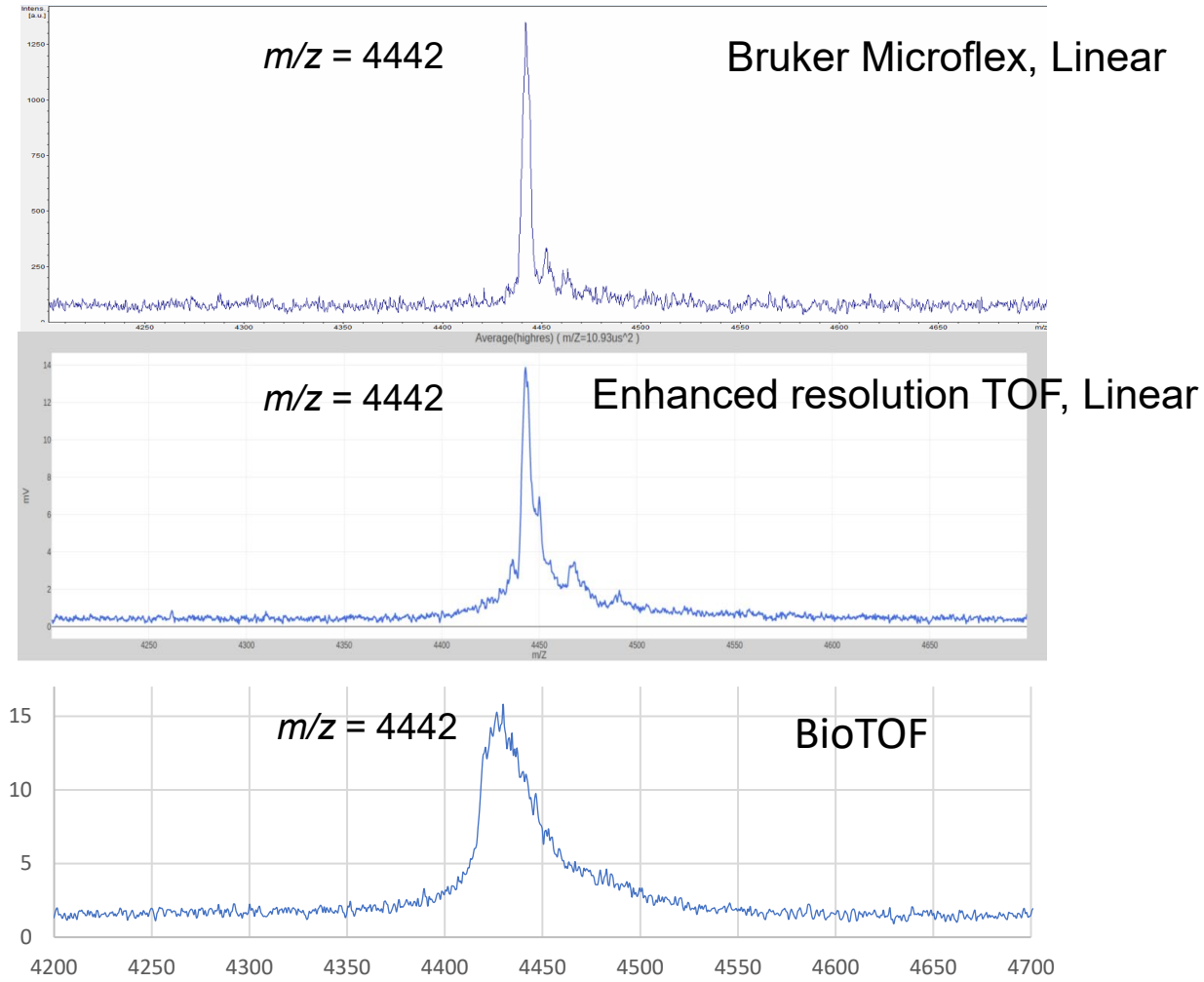
- The spectra display an extensive set of *E. coli* biomarkers at *m/z* = 2168, 2782, 3592, 4068, 4352, 5194, 5560, 7180, 8277 and 8690, which correspond to small, low molecular weight ribosomal proteins
- The spectrum recorded on enhanced resolution TOF is comparable to the spectrum recorded on Microflex

Mass spectra of *Bacillus globigii* (Bg) acquired on different systems



- The spectrum recorded on enhanced resolution TOF is comparable to the spectrum recorded on Microflex and significantly better when the spectrum acquired on BioTOF, which barely shows coat protein peaks.

Mass spectra of *m/z* = 4442 of Bg acquired on different systems



- Enhanced resolution TOF demonstrates mass resolving power comparable to Microflex and significant improvement over BioTOF.

Conclusions

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- A portable (0.5 m total length) MALDI-TOF mass spectrometer (enhanced resolution TOF) with two-stage acceleration, reflectron and delayed extraction was designed, built and tested.
- The enhanced resolution TOF can be operated either in linear or reflectron mode, simply by switching voltage on the reflectron.
- The system performance was compared with commercial MALDI-TOF instruments (Bruker Microflex and BioFlyte BioTOF) using large protein (BSA) and simulants (MS2, *E. coli* K12, Bg) samples.
- Enhanced resolution TOF demonstrated performance comparable to Microflex in linear mode and significantly better performance than BioTOF.

The authors gratefully acknowledge the support of the Army Research Office, contract # W911NF-17-C-0060.