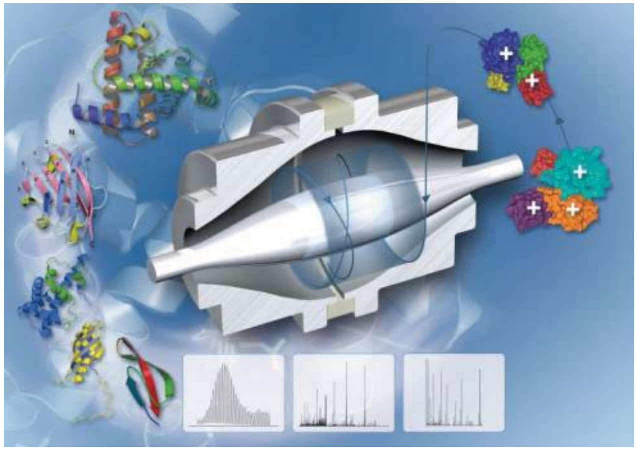
<u>Symposium:</u> ***New Alternatives in High-Resolution Mass Spectrometry***

Orbitrap Mass Spectrometry: Ultra-high Resolution for Every Lab

Alexander Makarov

March 14, 2012



The world leader in serving science

making connections
winning together

What is Orbitrap™ analyzer?

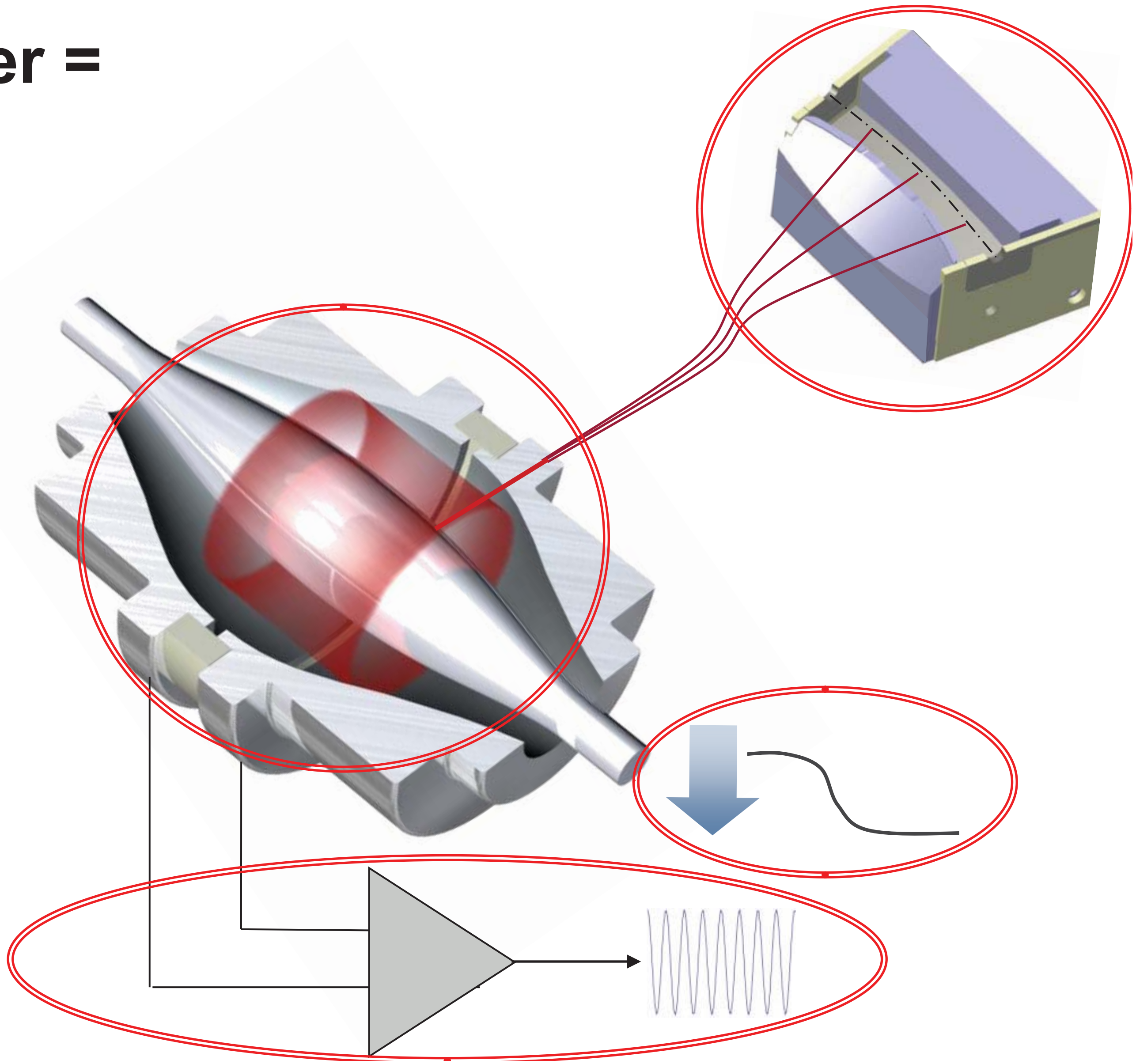
Orbitrap analyzer =

= Orbital
trapping

+ Image current
detection

+ Electrodynamic
squeezing

+ External pulsed
ion source

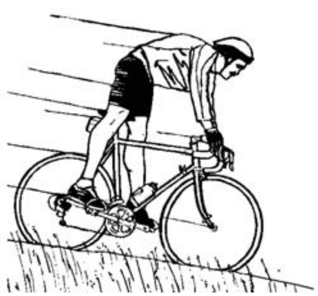


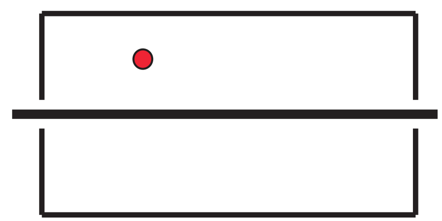
The unstable world of electrostatic trapping

Earnshaw's theorem (1842):

"A collection of point charges cannot be maintained in a stable stationary equilibrium configuration solely by electrostatic interaction of the charges"

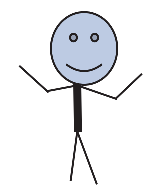
…but ***moving*** charges could be stable!





Orbital traps
Kingdon (1923)

No promise?

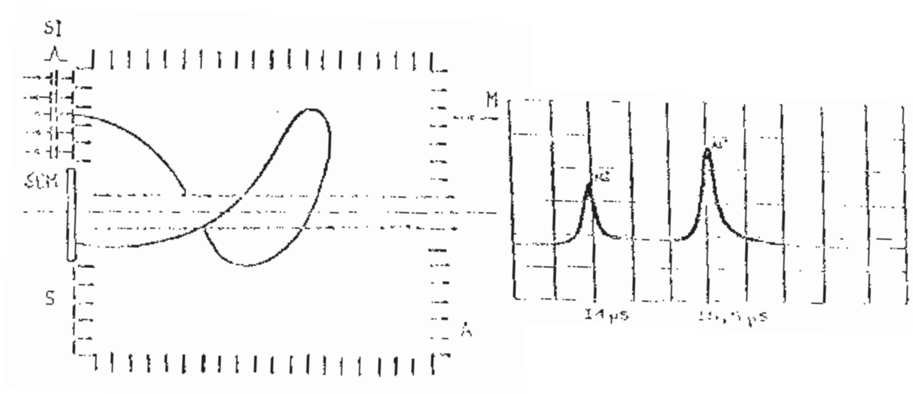


TOF Adventures: Prelude to the Orbitrap analyzer

$$U(r,z) = \frac{k}{2} \cdot \left\{ z^2 - r^2/2 + R_m^2 \cdot \ln(r/R_m) \right\}$$

Gall L.N., Golikov Y.K., Aleksandrov M.L.,
Pechalina Y.E., Holin N.A. *SU Pat. 1247973*, 1986.

1st implementation of quadro-logarithmic potential in mass spectrometry (1989)

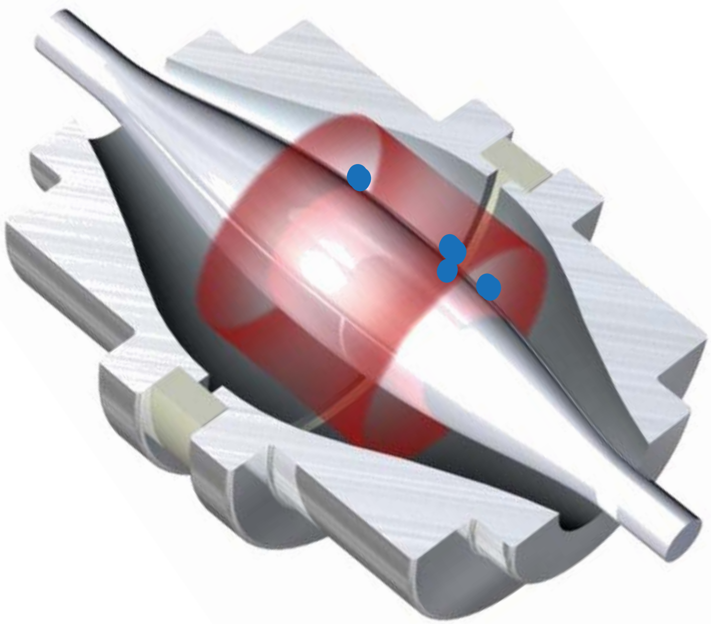


"A mosquito-catcher"

Orbital trapping

"Ideal Kingdon trap":
Quadro-logarithmic potential

$$U(r,z)=\frac{k}{2}\cdot\left\{z^2-r^2/2+R_m^2\cdot\ln(r/R_m)\right\}$$



"Beauty will save the world."- F.M. Dostoevsky

Characteristic frequencies:

- Frequency of rotation ω_φ
- Frequency of radial oscillations ω_r
- Frequency of axial oscillations ω_z

$$\omega_\varphi=\frac{\omega_z}{\sqrt{2}}\sqrt{\left(\frac{R_m}{R}\right)^2-1}$$

$$\omega_r=\omega_z\sqrt{\left(\frac{R_m}{R}\right)^2-2}$$

$$\boxed{\omega_z=\sqrt{\frac{k}{m/q}}}$$

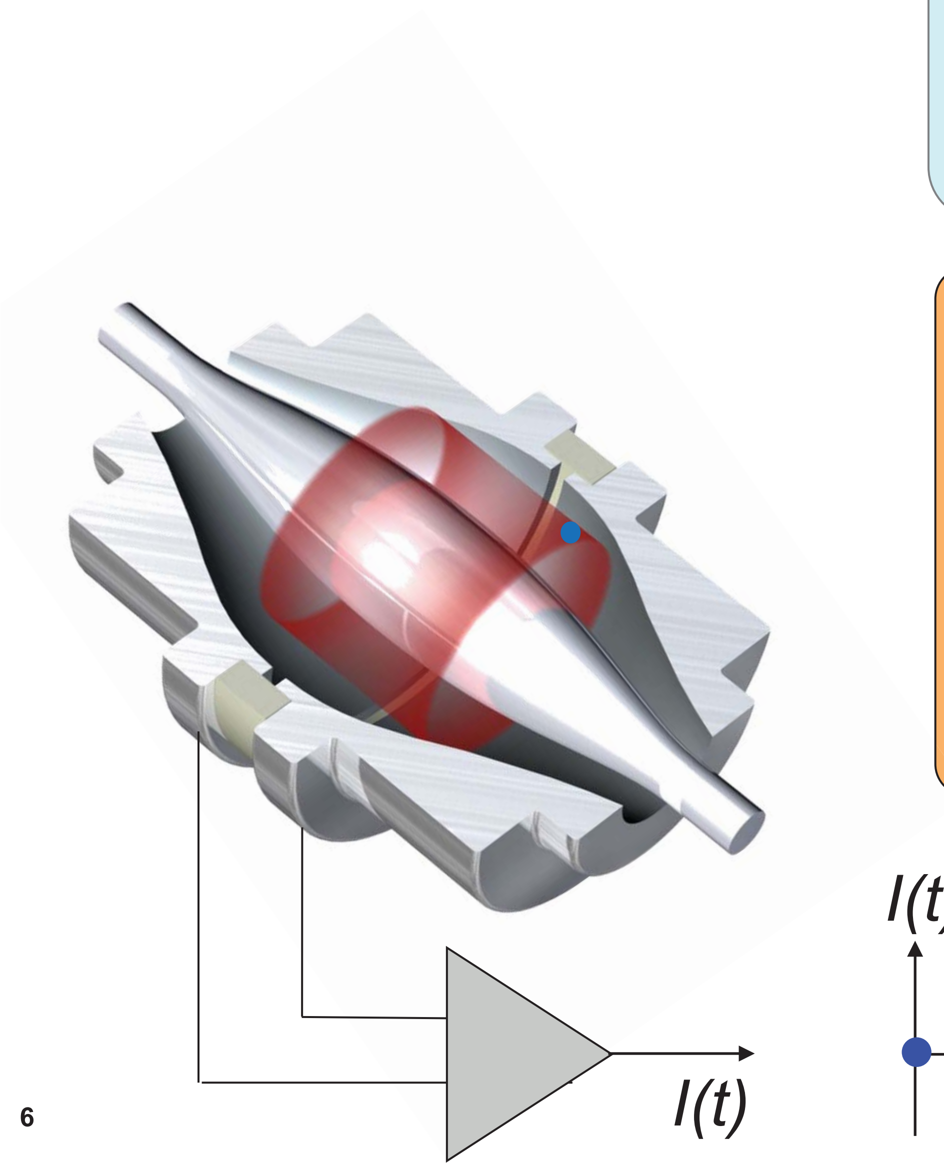
Only this frequency does not depend on energy, angle, etc. and is used for mass analysis

Detection of Ions in the Orbitrap analyzer

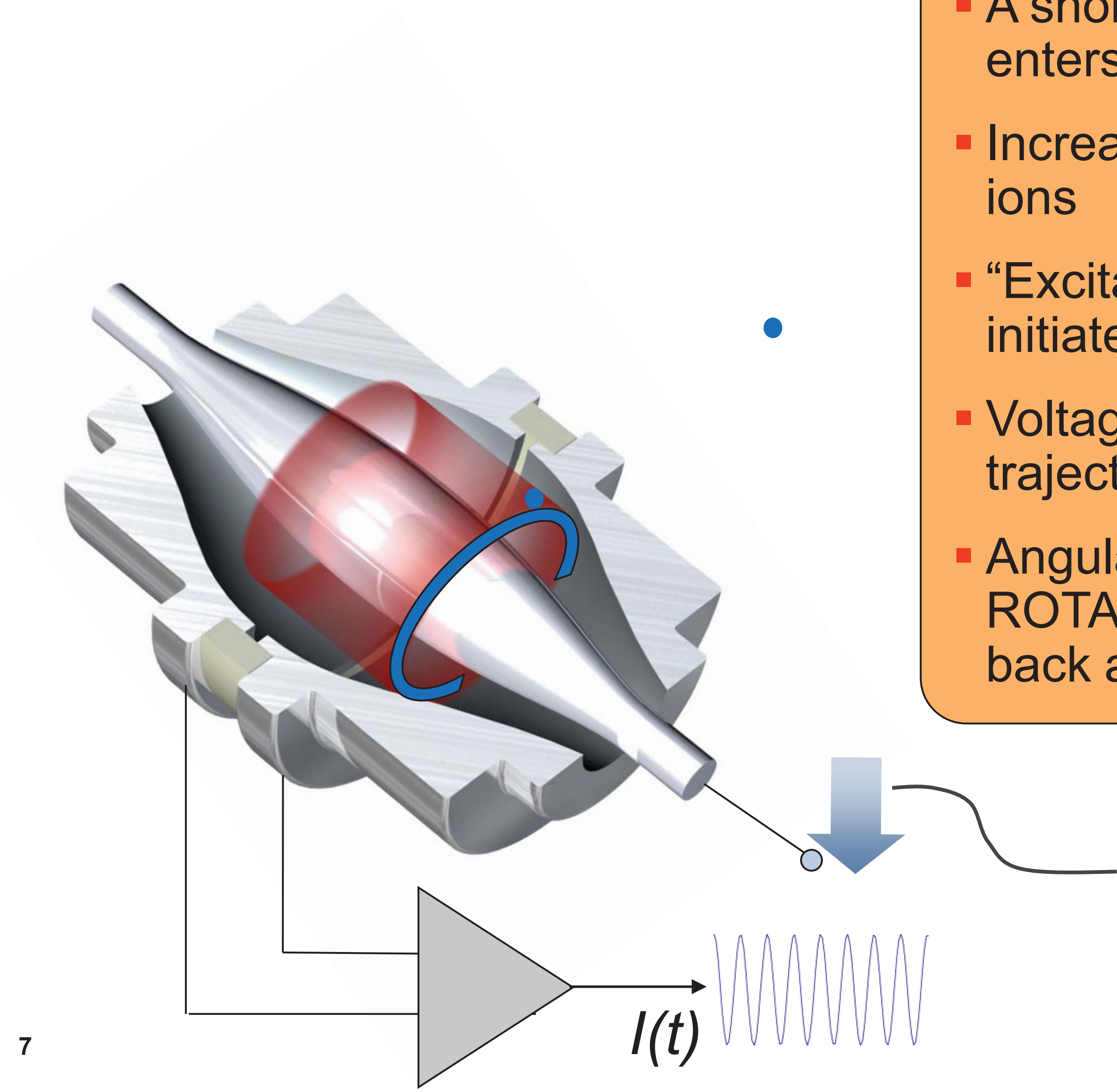
Image current detection

- All-mass detection
- Noise equivalent to <10 ē/√Hz

- Frequency of axial oscillations of each ring induces an image current on <u>split</u> outer electrodes
- Multiple ions in the Orbitrap generate a complex signal with frequencies determined using a Fourier Transformation

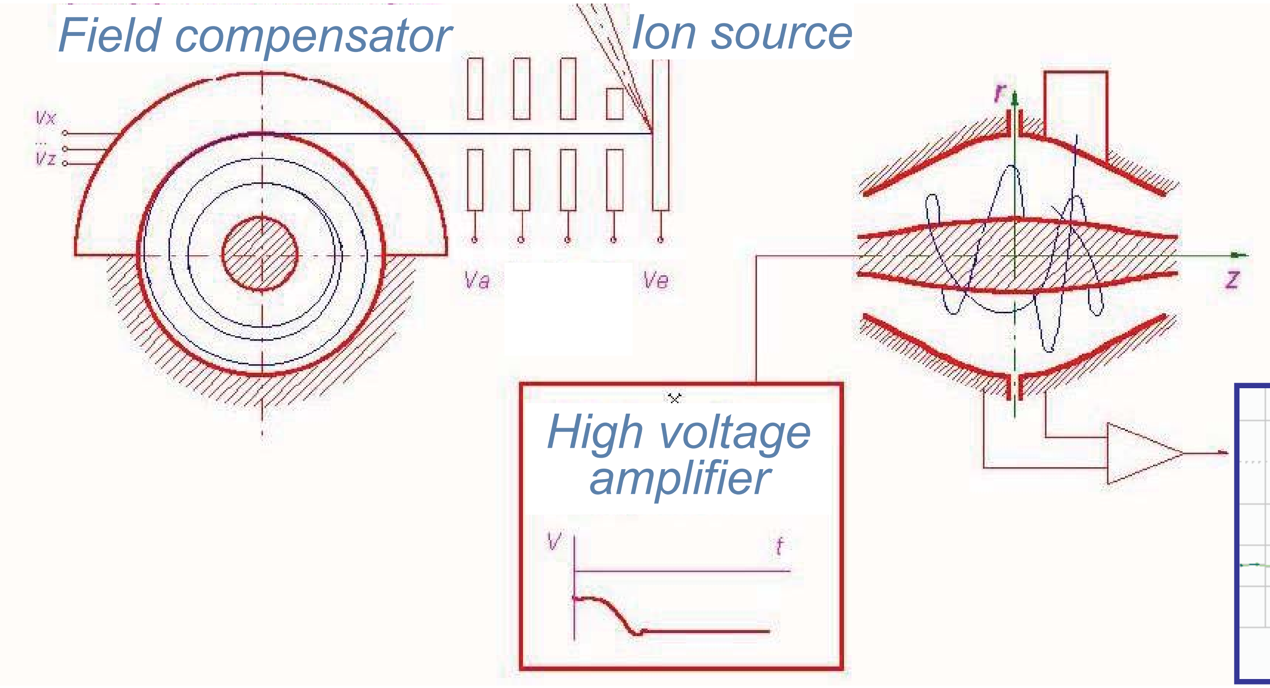


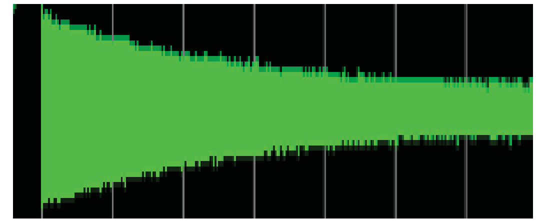
Injection of Ions into the Orbitrap analyzer

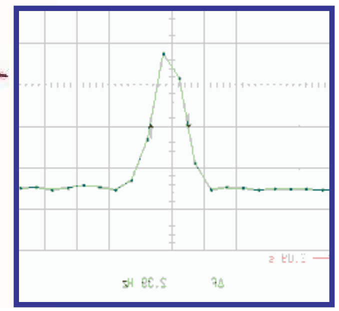


- A short ion packet of one m/z enters the field
- Increasing voltage squeezes ions
- “Excitation by injection” is initiated
- Voltage stabilises and ion trajectories are also stabilized
- Angular spreading forms ROTATING RINGs bouncing back and forth

Proof of Principle: Orbitrap MS with Laser Ion Source







8 M record length
10 Ms/s (borrowed LeCroy)
0.8 s transient
f =711 kHz,Δf=2.39 Hz
f/Δf≈ 300000
M/ΔM=½ f/Δf ≈ 150000

HD Technologies Ltd
From Jan. 25, 2000- within Thermo Inc.
*(in **Thermo Masslab**, Manchester, UK)*

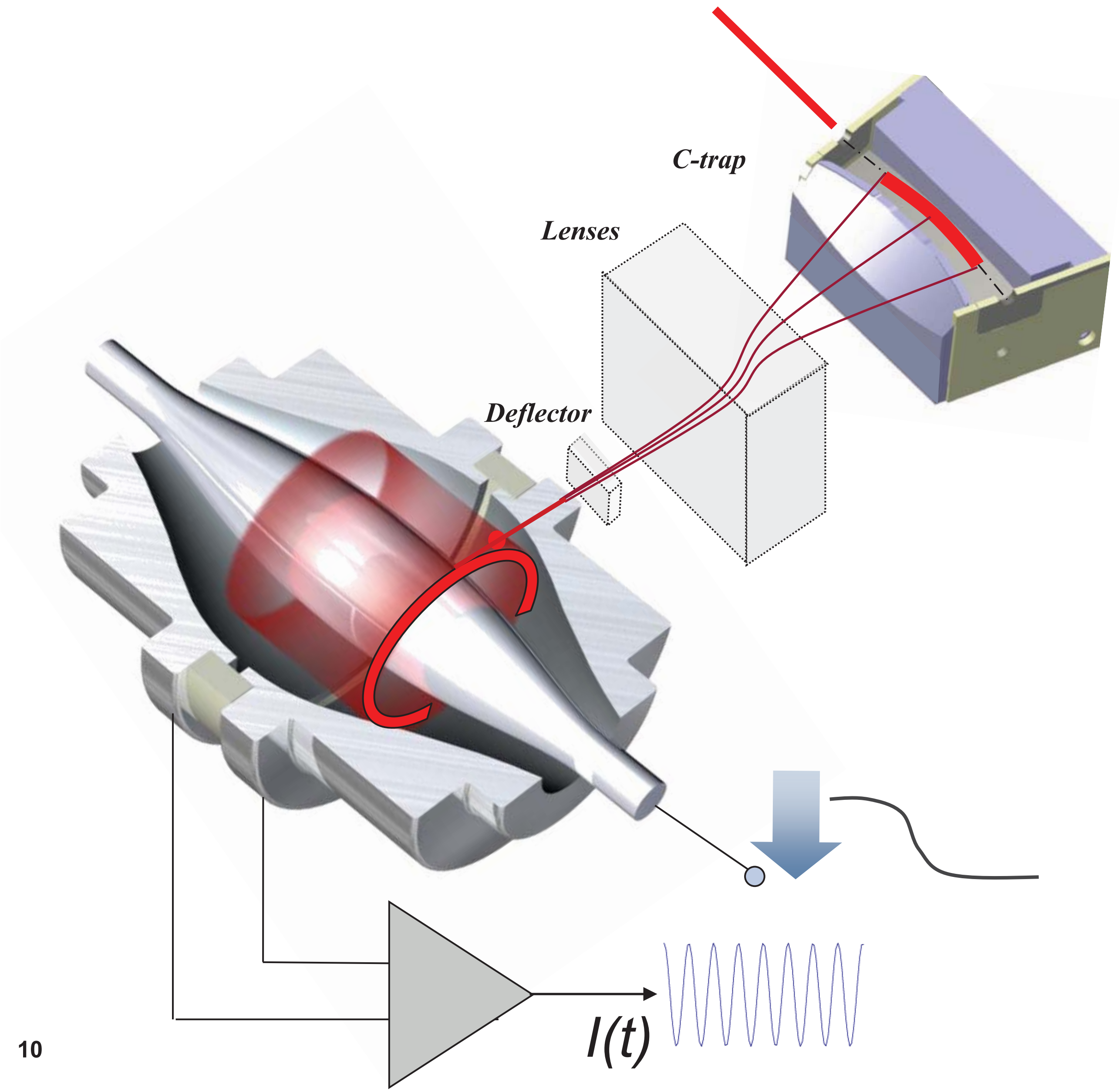
A.A. Makarov, *Anal. Chem., v.72 (2000), No.6, p.1156-1162.*

Reasons Why Orbitrap Would Never Work

- Not possible to provide ion packets with required spatial and temporal parameters for continuous ion sources
- Tolerance requirements on electrodes are not realistic
- Injection and central slots ruin resolving power and mass accuracy
- Vacuum requirements are ridiculously high and can not be met
- Ions can not be injected with high efficiency
- Wide mass range can not be injected and captured
- Image current preamplifier will be destroyed by pick-up during injection
- Noise from high voltage power supply will overwhelm preamplifier
- Surface potentials would disturb and scatter ions
- Mass accuracy will be poor because of voltage drift & noise
- Large ion numbers cannot be properly injected or analyzed
- Electrodes shape, rotational and radial frequencies will cause unmanageable mass-dependent harmonics

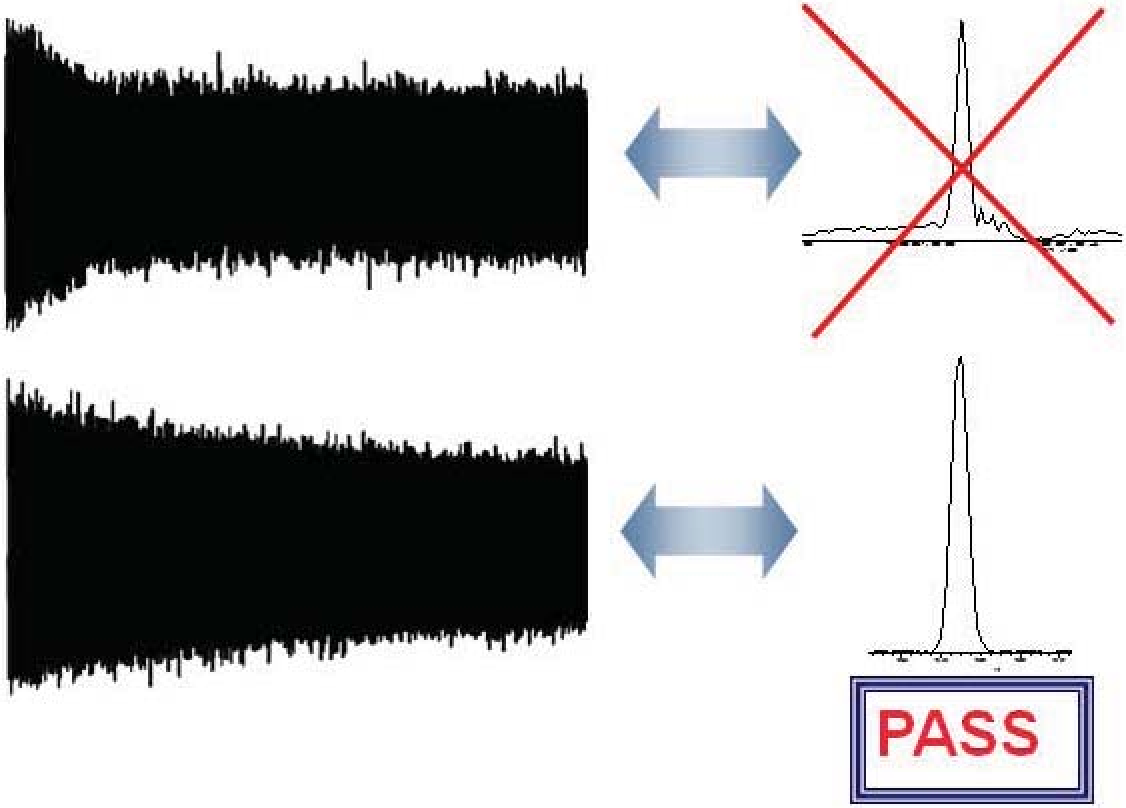
All these reasons are valid and require a lot of work!

Injection of Ions into the Orbitrap analyzer



- Ions are stored and cooled in a curved RF-only quadrupole (C-trap)
- RF is ramped down, radial DC is applied
- Ions are ejected along lines converging on the orbitrap entrance).
- As ions enter orbitrap, they are picked up and squeezed by its electric field
- All ions start simul-taneously, but light ions enter Orbitrap analyzer earlier that heavy ions

Influence of (in) Correct Electrode Shape

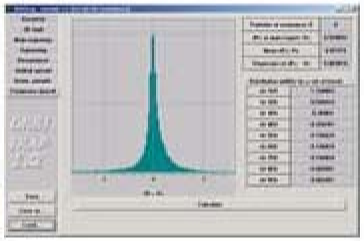


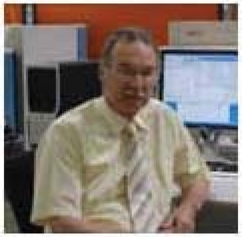
De-focusing +
self-bunching =
bad peak shape

Correct tolerances =
High resolution &
good peak shape

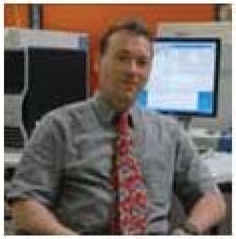
M. Monastyrski

D. Grinfeld





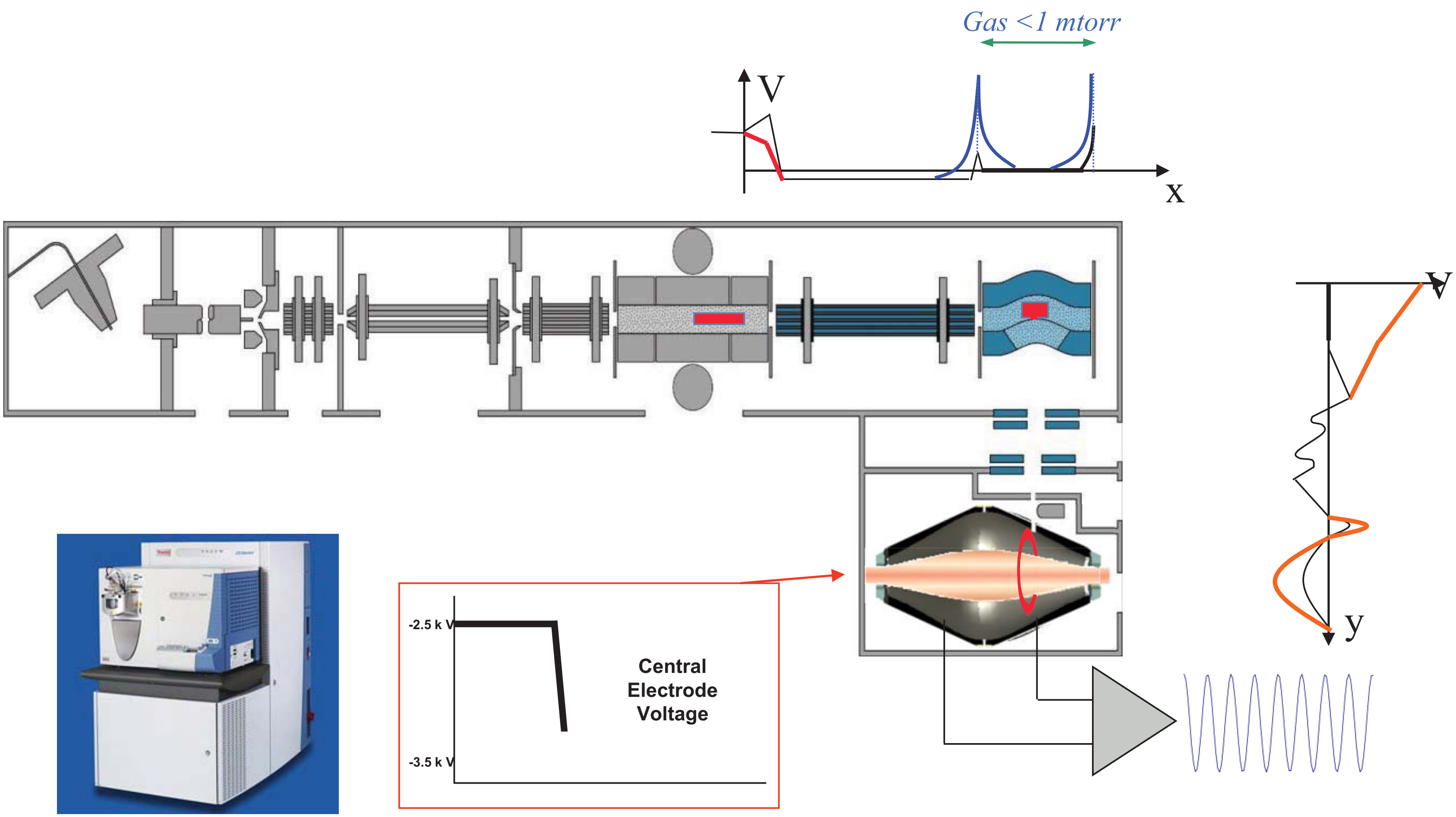
B. Laser



F. Schaefer

LTQ-Orbitrap: All Technologies Come Together

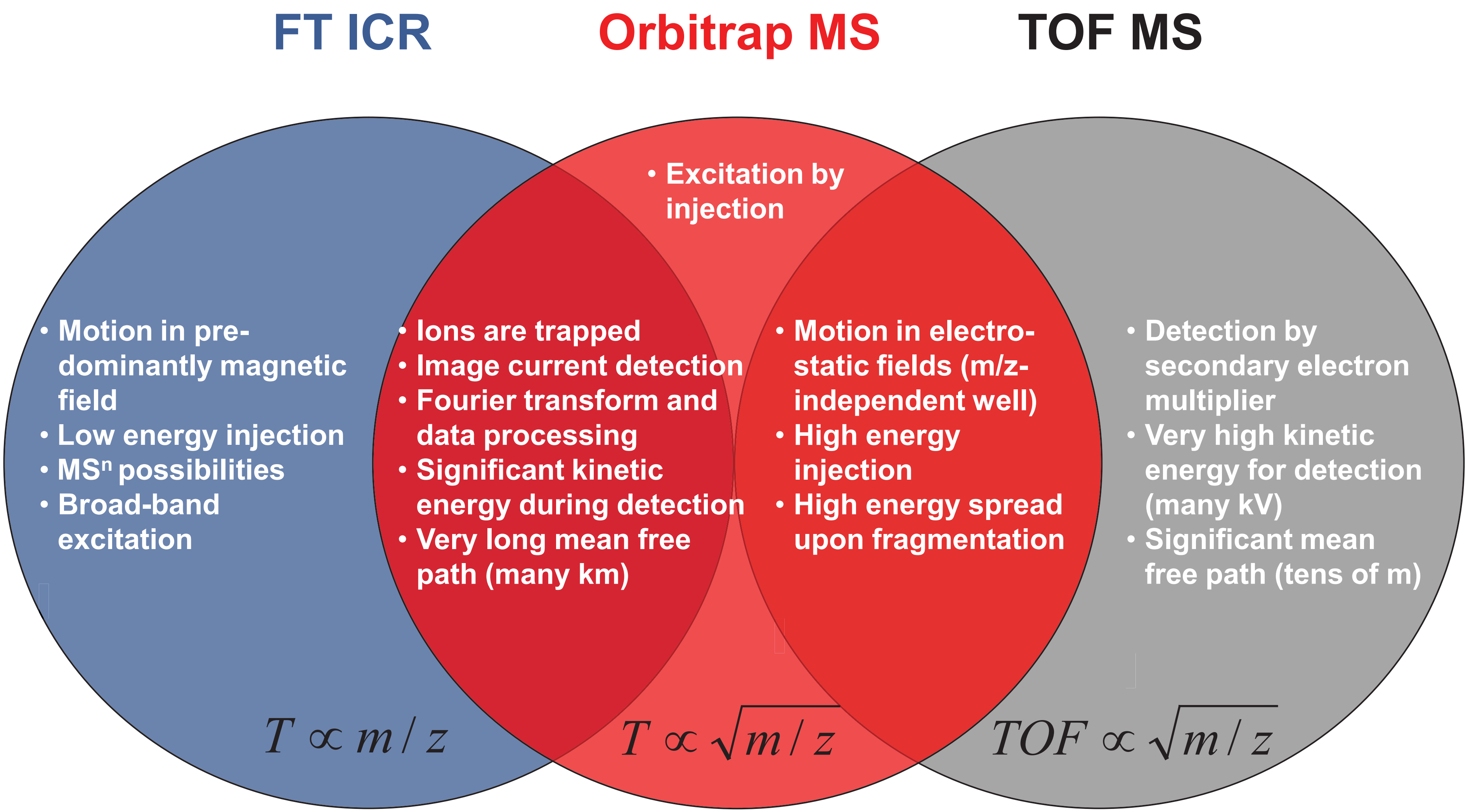
1. **Ions are stored in the linear trap of LTQ**



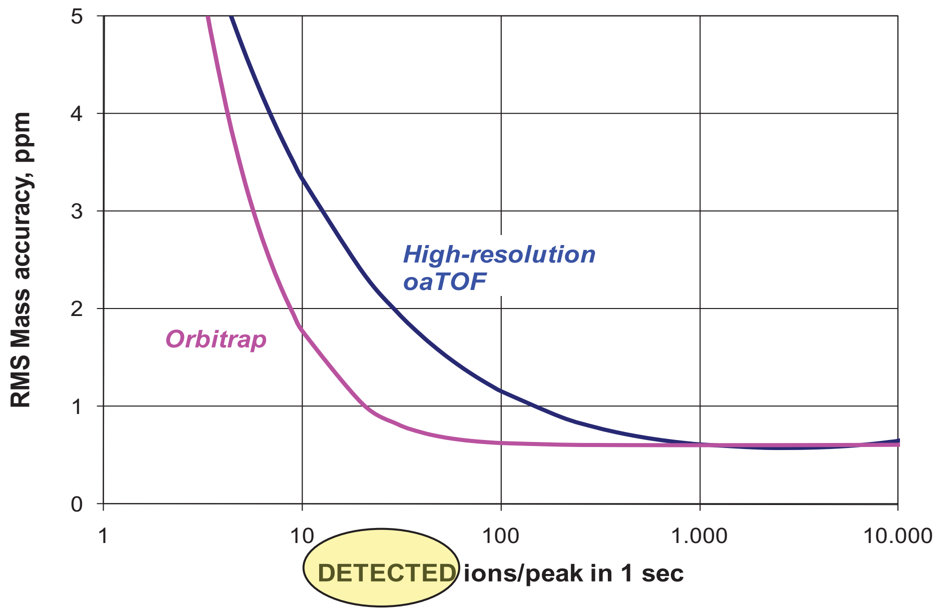
 LTQ Orbitrap™ 2005

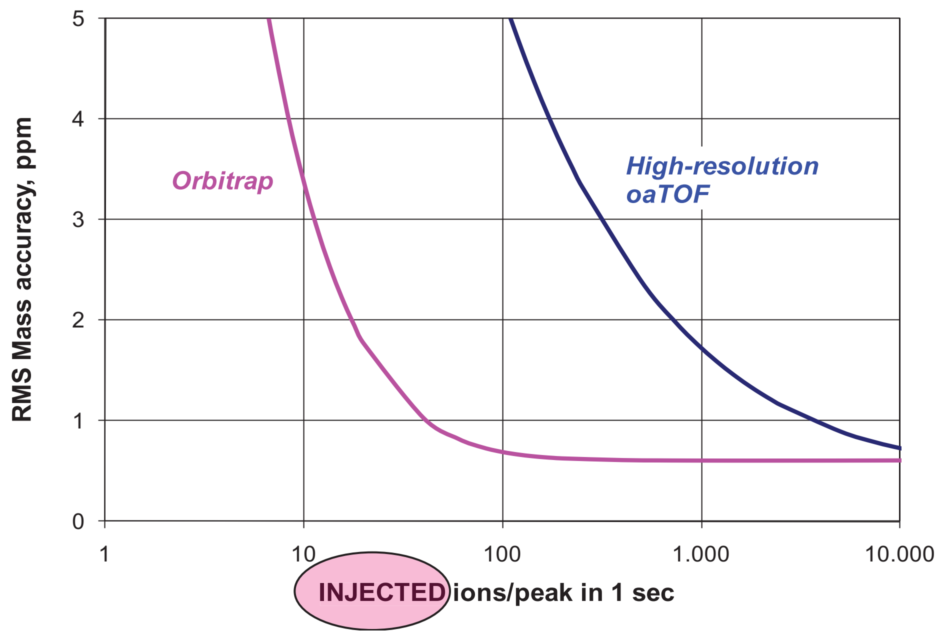
Transmission=30..50%

Major accurate-mass analyzers for Life Science



Importance of high transmission

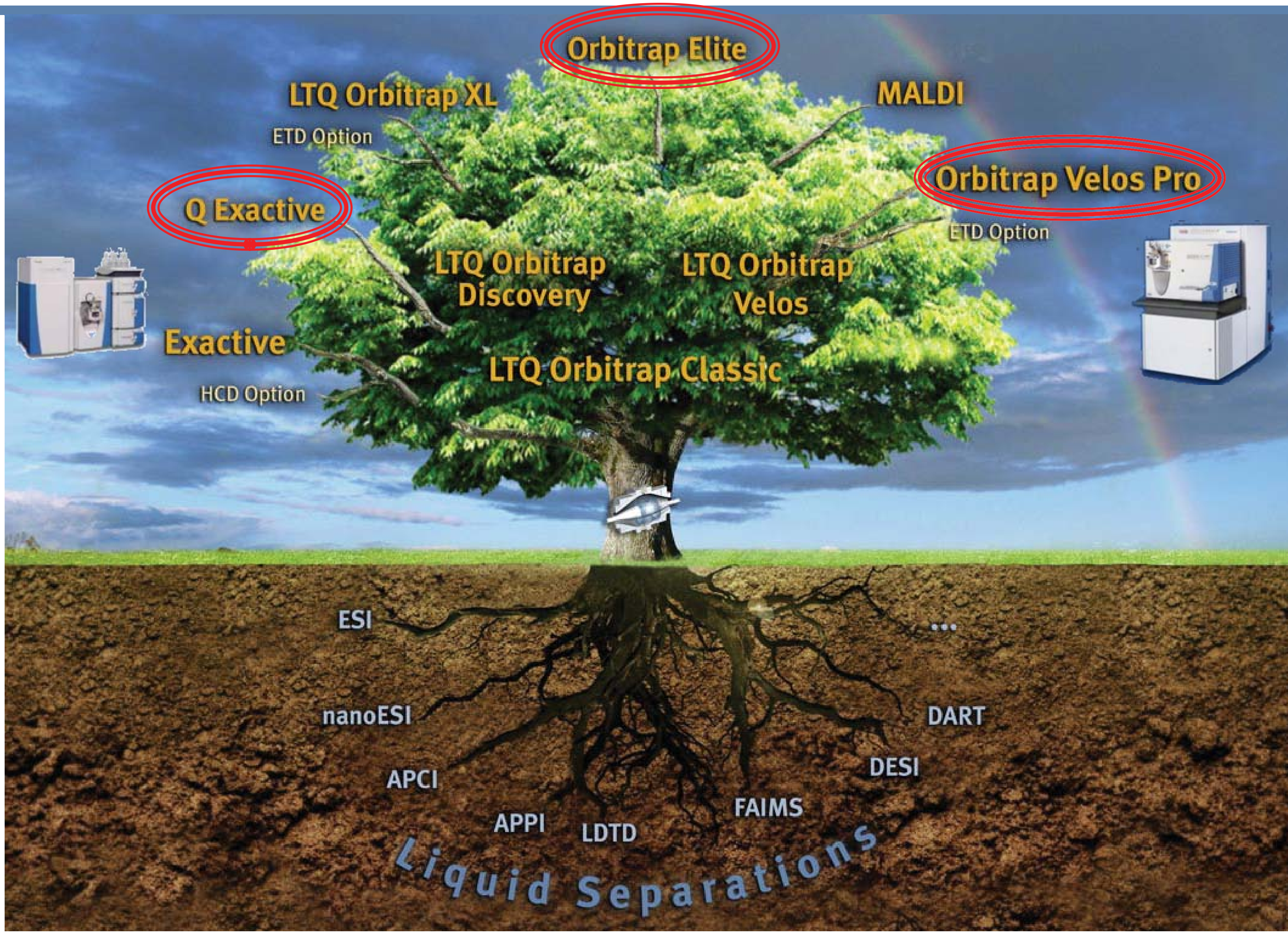




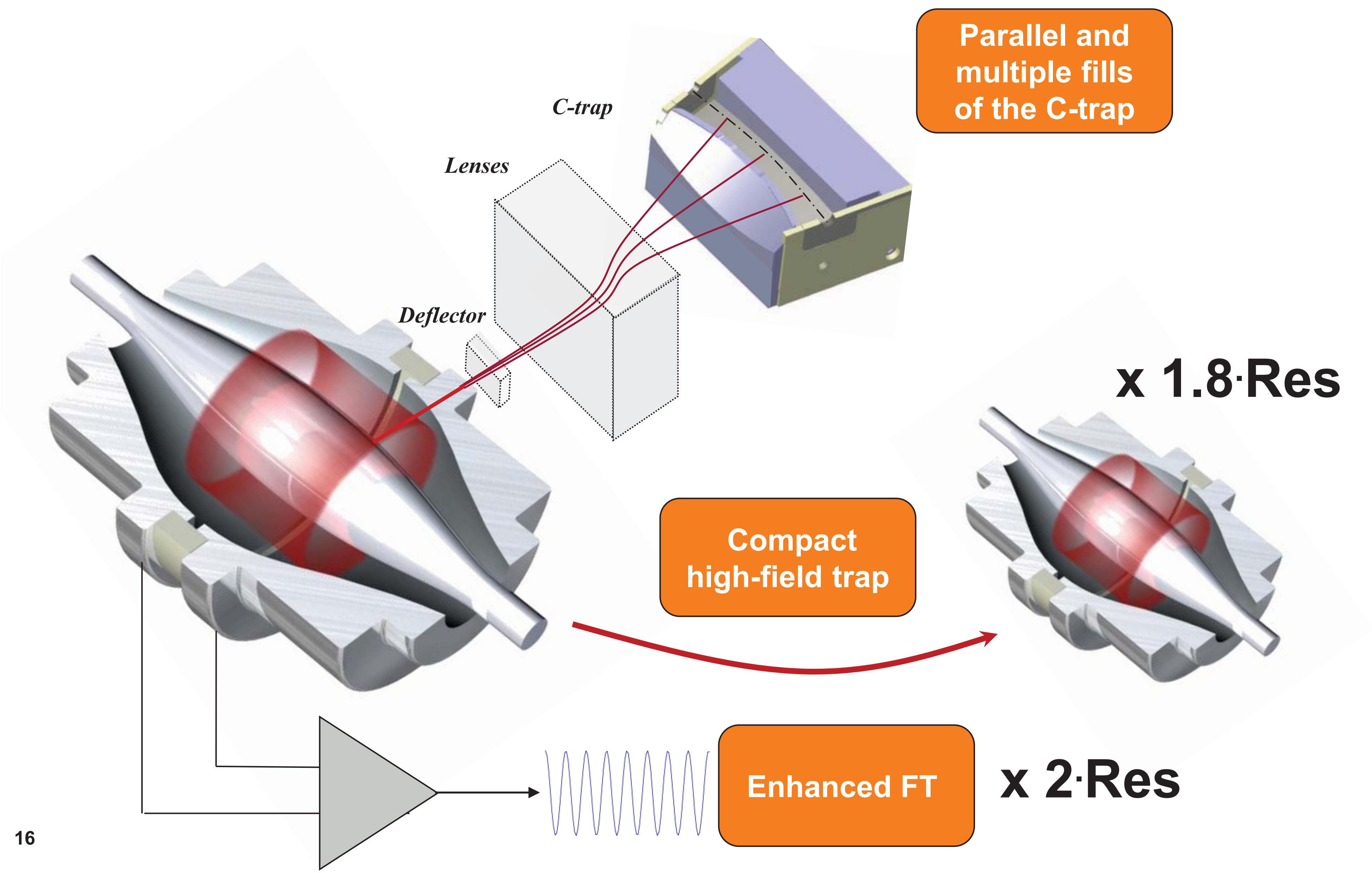
Assumptions:

- oaTOF: resolving power 40,000, transmission 4% (grids x duty cycle x angular scattering on grids)
- Orbitrap transmission 50%

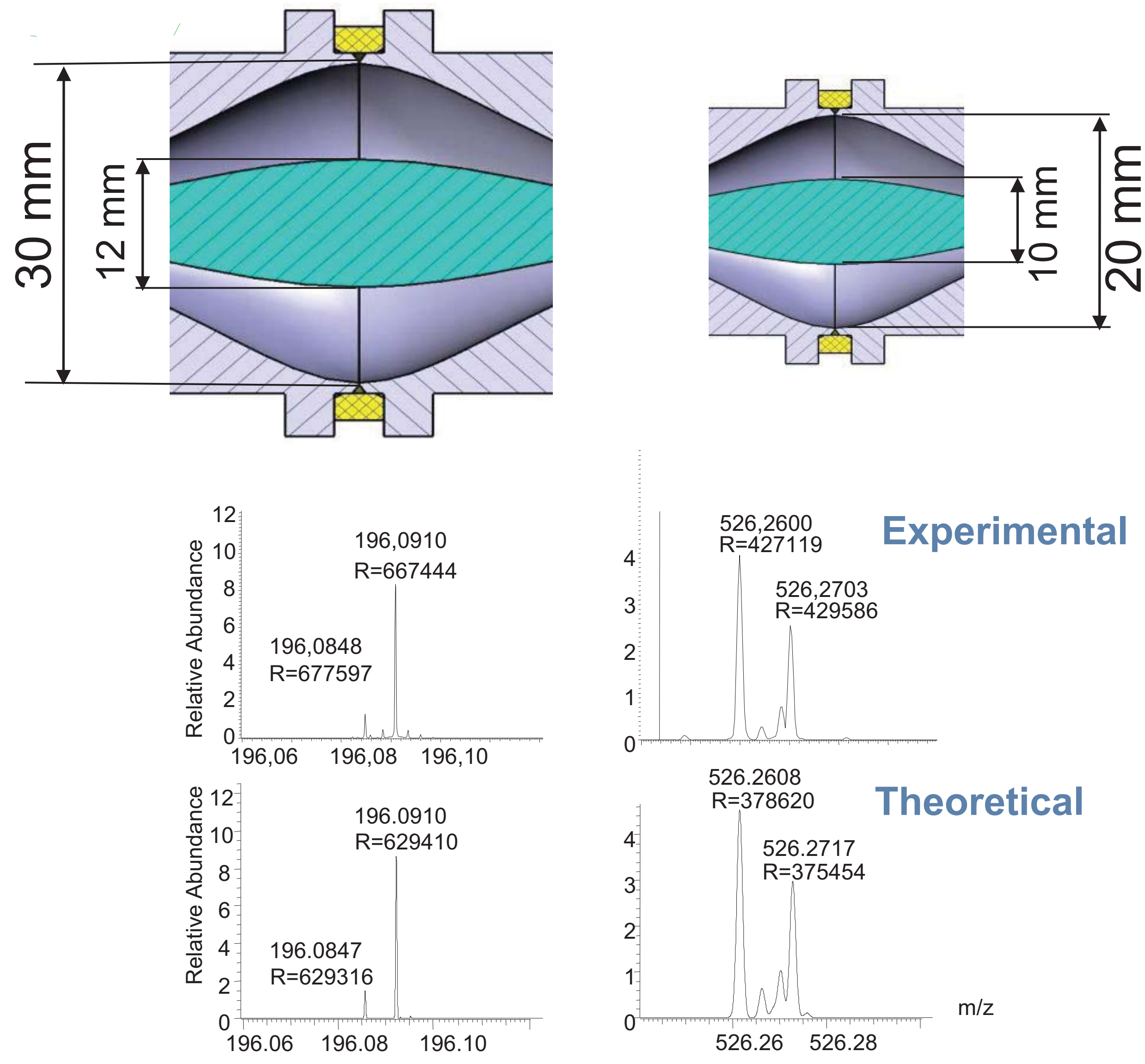
2011: A new season in life of Orbitrap mass spectrometry



Recent developments of the Orbitrap analyzer



Improvements in orbital trapping: Compact high-field analyzer



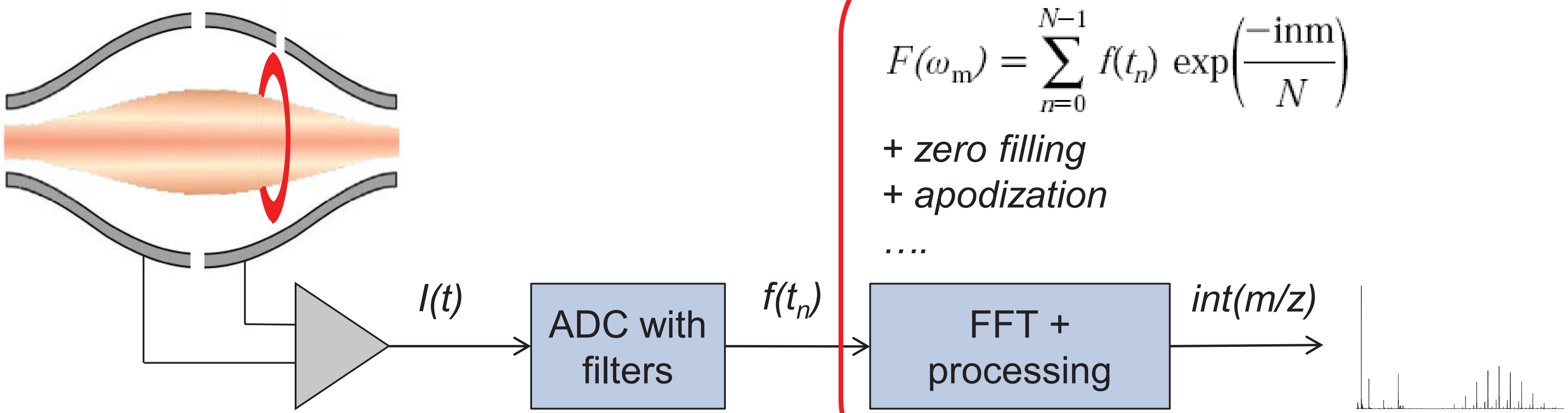
- Smaller size- 1.8x frequency at the same voltage, 2.1x at higher voltage
- New miniature lenses for focusing onto Orbitrap entrance
- Higher tolerance requirements
- Lower capacitance and new preamplifier transistors bring increased sensitivity.
- Space charge shift: ca. 70% rel. to the standard trap at the same target

Detection process in the Orbitrap analyzer

$$F(\omega_m) = \sum_{n=0}^{N-1} f(t_n)\, \exp\left(\frac{-\mathrm{inm}}{N}\right)$$

+ *zero filling*
+ *apodization*

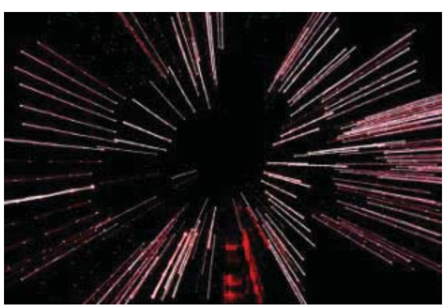
….



All ions are ejected from the C-trap at the same moment

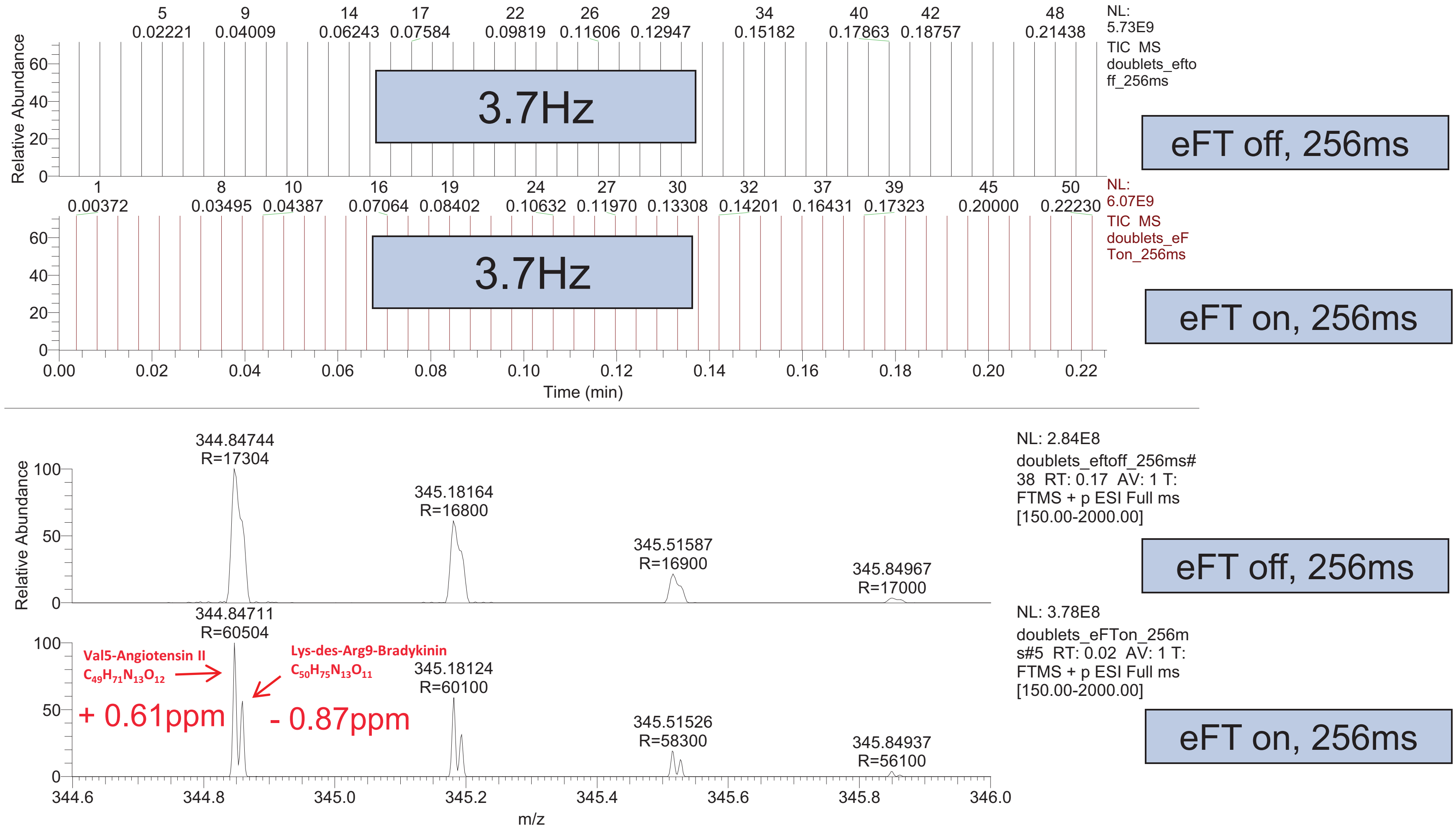
Coherent nature of ion packets allows for a new advanced signal processing procedure

Narrow peaks in *Enhanced FT*



Enhanced FT as a tool for increasing resolving power

- MS analysis of two peptides (Val5-Angiotensin II and Lys-des-Arg9-Bradykynin) differing in mass by 12.1 mmu (triply charged ions)

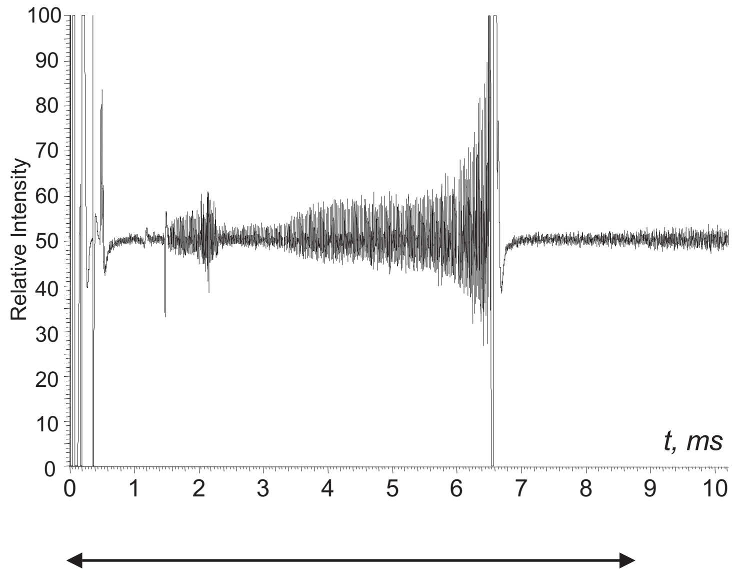


Please see: O. Lange; E. Damoc; A. Wieghaus; A. Makarov. “Enhanced FT for Orbitrap Mass Spectrometry”. Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011.

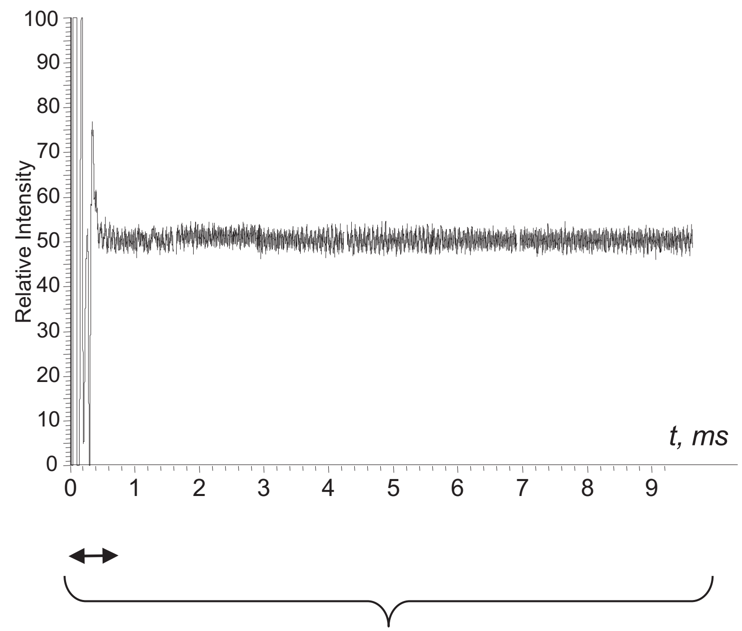
Enhanced FT is not just a software!

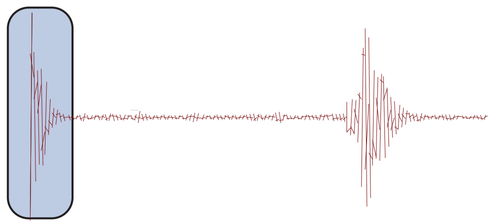
- Improved stabilization and filtering of voltages
- Special dielectric materials
- Improved preamplifier

Before



After

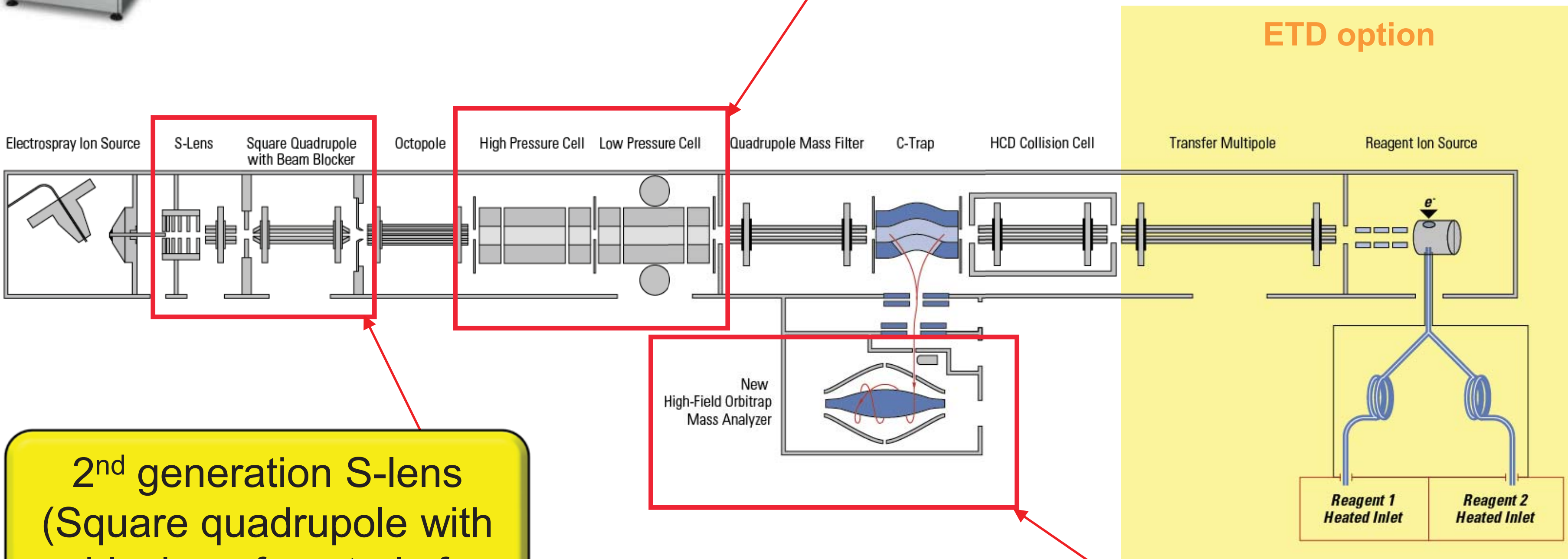




<u>Conclusion:</u> detection of the first beat is very important for heavy intact proteins

Orbitrap Elite instrument

Faster Dual trap electronics: >12 scans/sec



2nd generation S-lens (Square quadrupole with a blocker of neutrals for improved ruggedness)

Compact high-field Orbitrap analyzer+ eFT

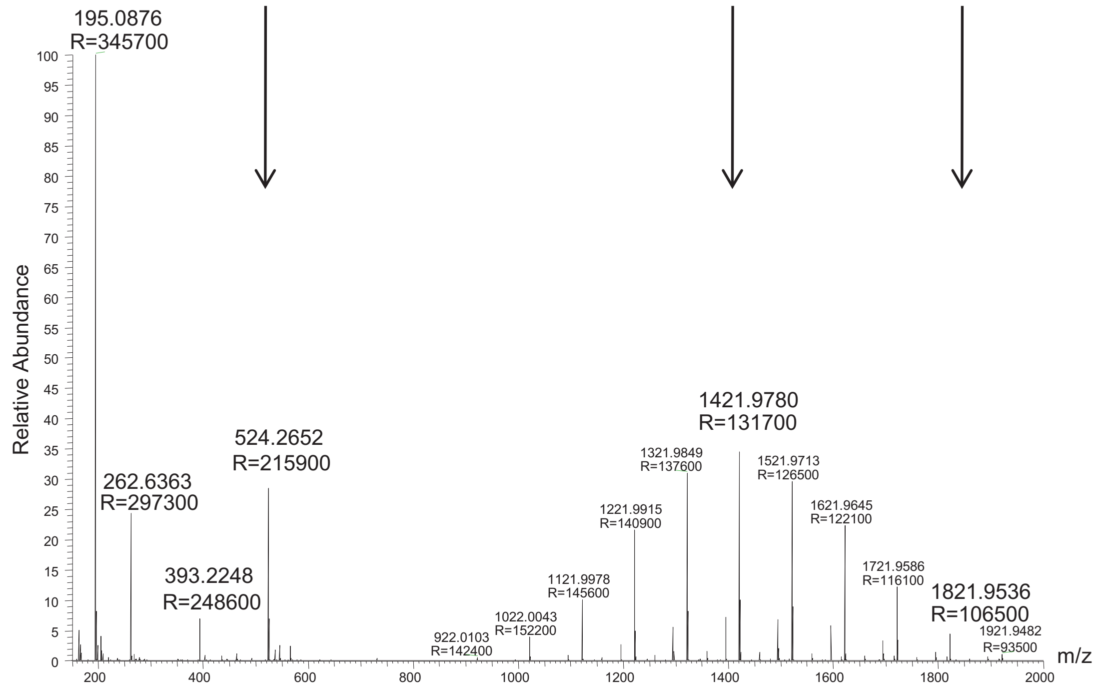
Resolving power of Orbitrap Elite

0.76 sec acquisition
(std for R=60,000)
Hamming apodization,
T=5e5

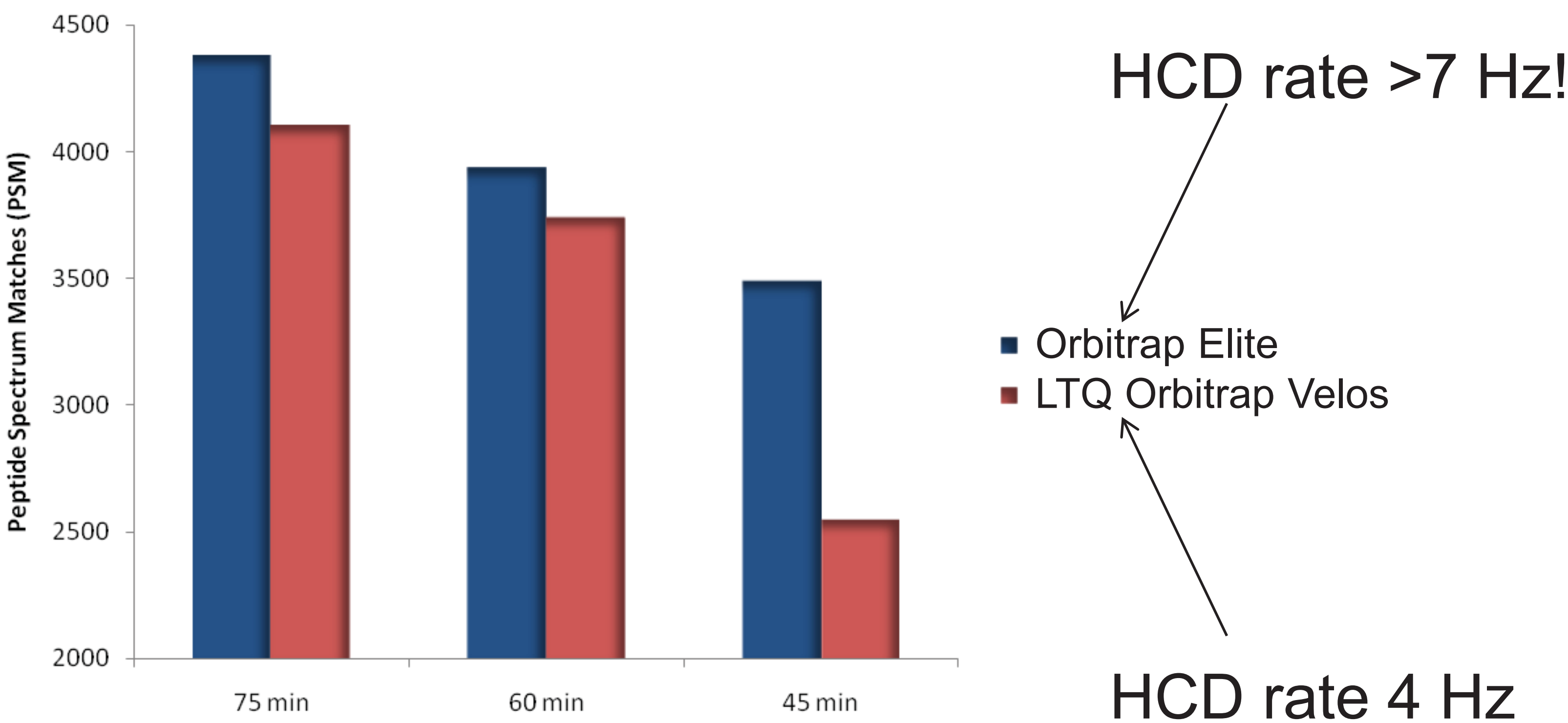
R=60,000 ⟷ 393

m/z	R_{FWHM} in 0.76s
195	345,000
393	248,000
524	215,000
1222	140,000
1822	106,000

LTQ FT equivalent: 12T 20T 32T 34T



Number of peptide spectrum matches for different LC gradients

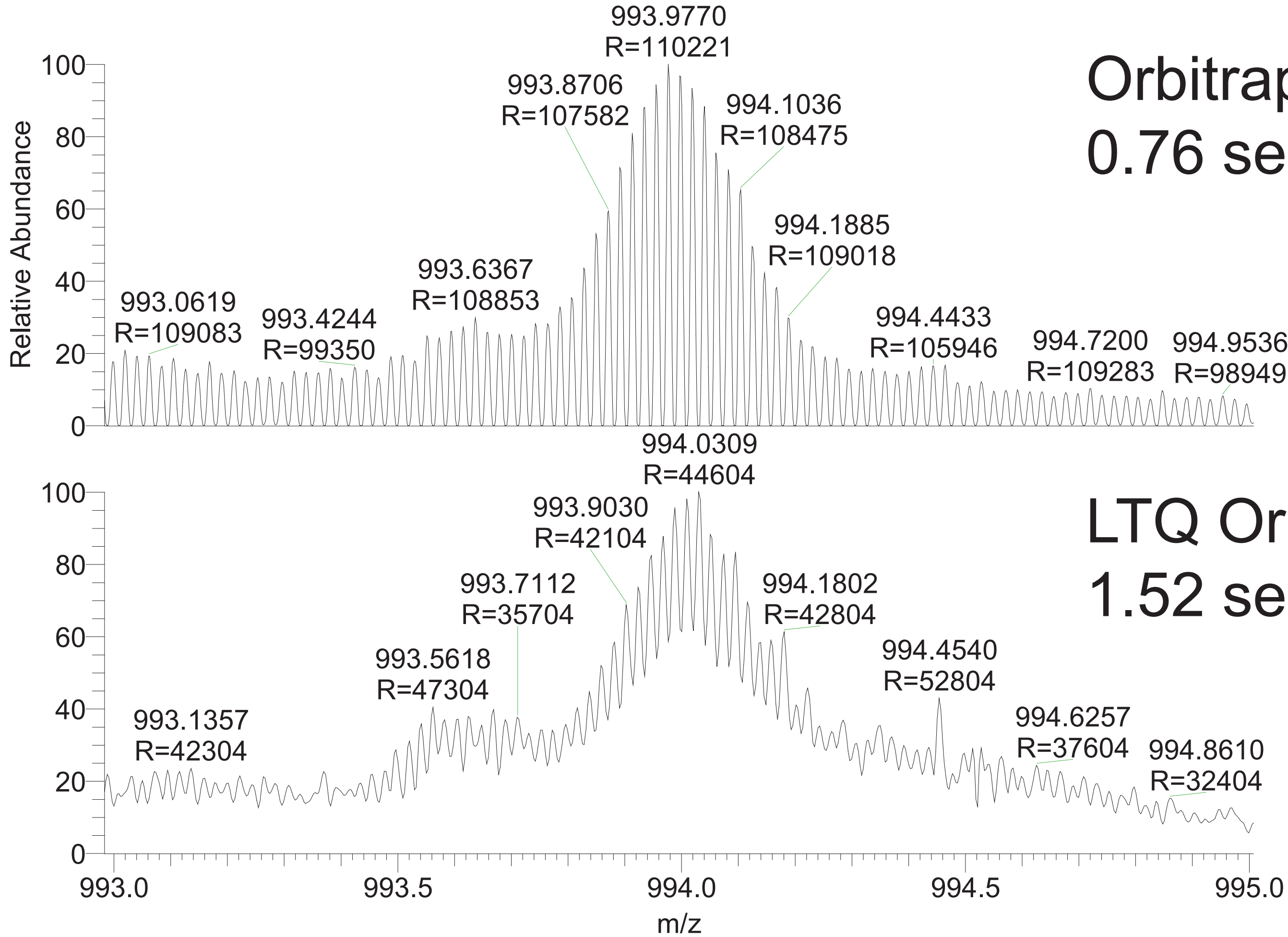


Sample: *E.coli,* HCD top 15 method

Slide courtesy
M.Zeller

Please see also: M. Zeller; C. Crone; M. Mueller; E. Damoc; E. Denisov; A. Makarov; D. Nolting; T. Moehring. “Increased analytical performance on a hybrid iontrap-FTMS mass spectrometer with a compact Orbitrap mass analyzer”, Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011.

Protein performance



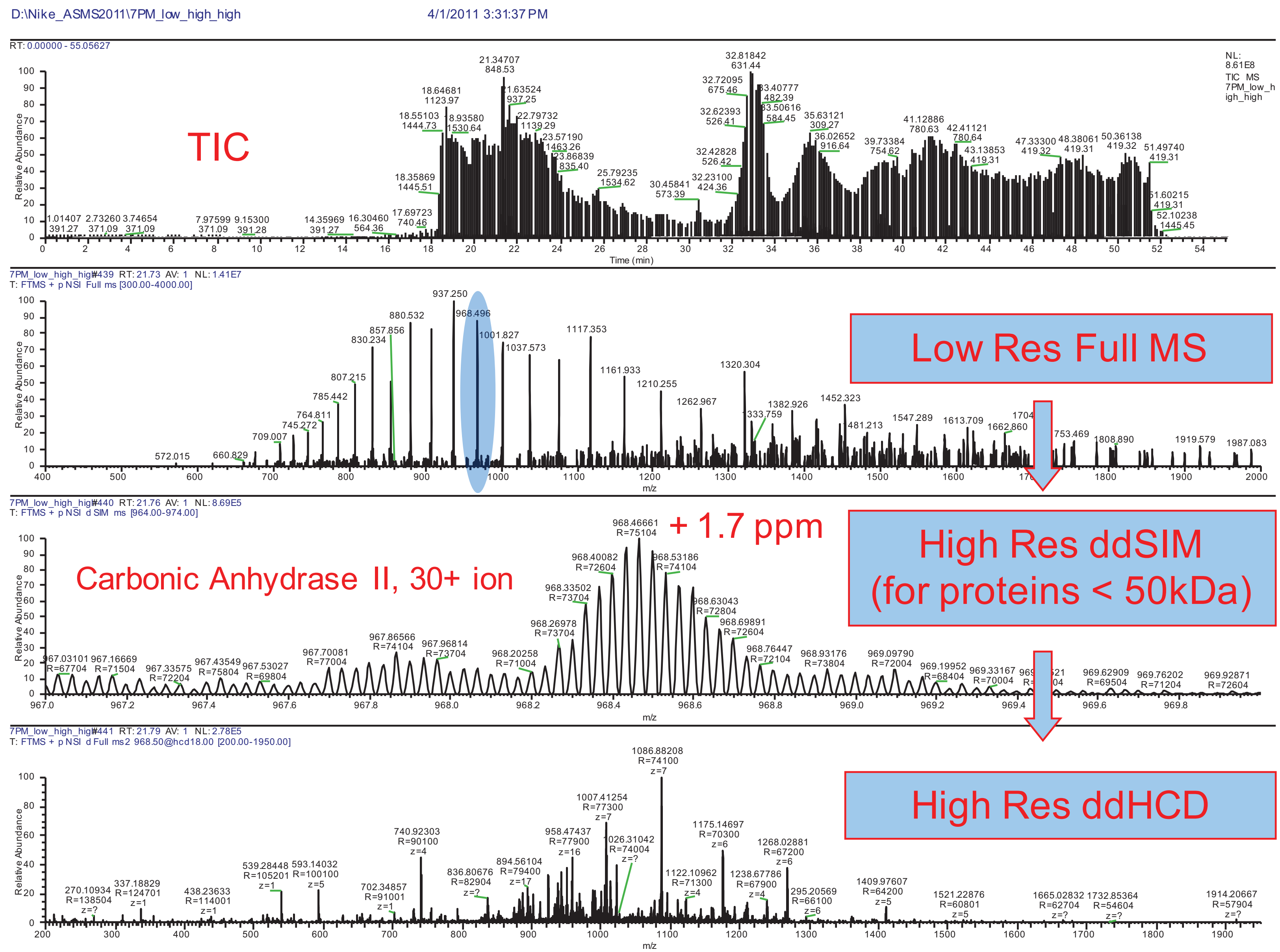
Slide courtesy
E. Damoc, M.Zeller

Intact Yeast Enolase (46.64 kDa), 47+ ion (P<1*10^{-10} Torr)

Please see also: E. Damoc, E. Denisov, O. Lange, T. Moehring, A. Makarov. "Improving protein analysis in Orbitrap mass spectrometry", Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011.

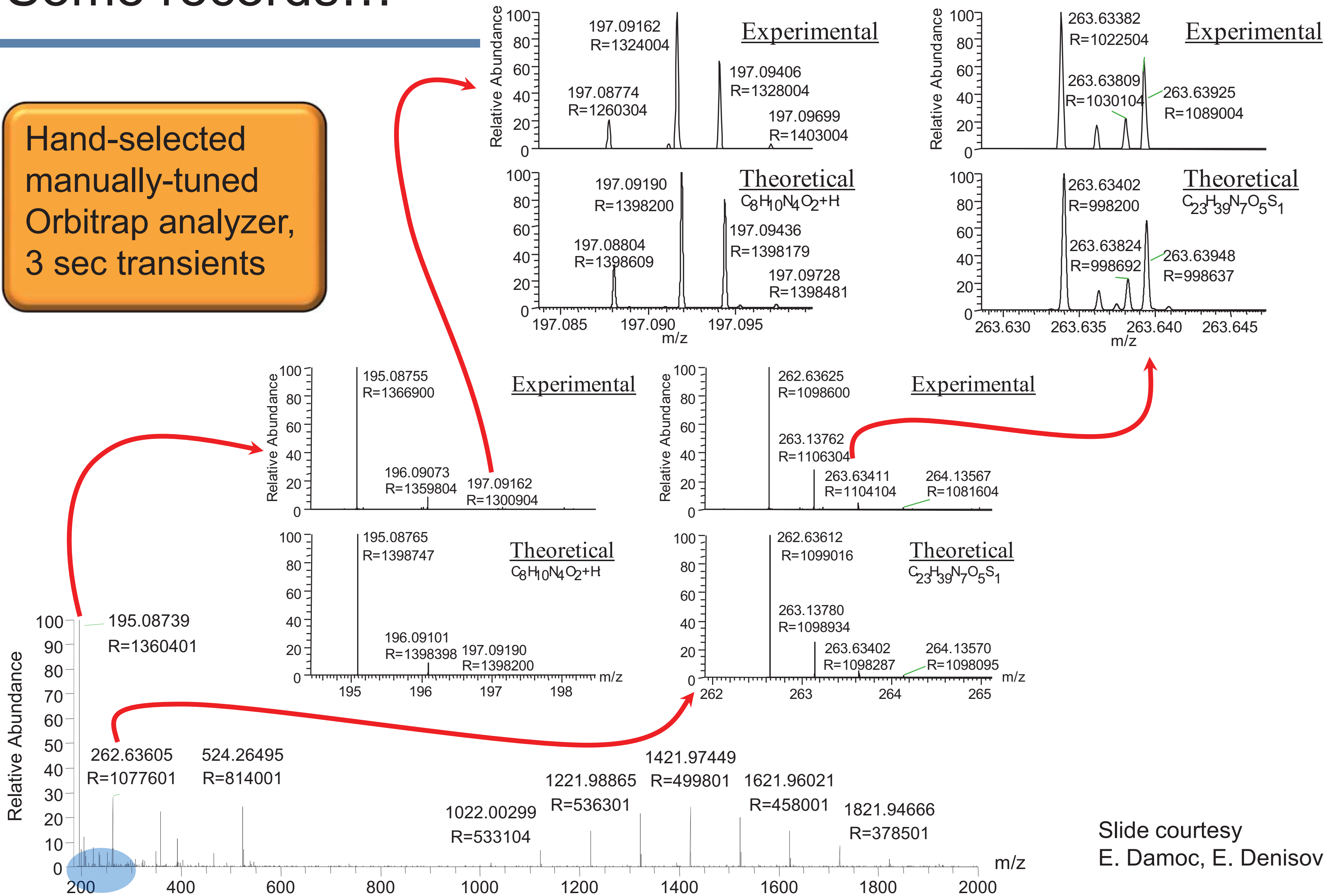
Please see also: P. Compton; E. Damoc; E. Denisov; J.C. Tran; A. Wieghaus; M.W. Senko; S.R. Horning; A.Makarov; N.L. Kelleher. "Top-Down Proteomics on Orbitrap-Based Mass Spectrometers" Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011.

One of workflows for top-down analysis



Some records…

Hand-selected manually-tuned Orbitrap analyzer, 3 sec transients

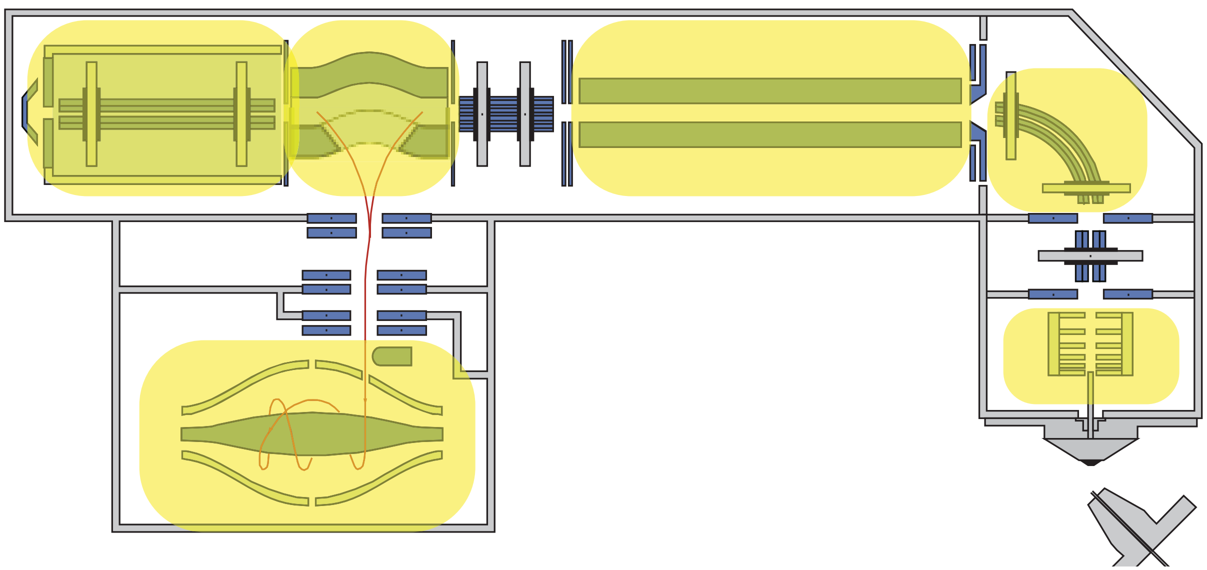


Slide courtesy
E. Damoc, E. Denisov

Research only!

Q Exactive: new features relatively to Exactive

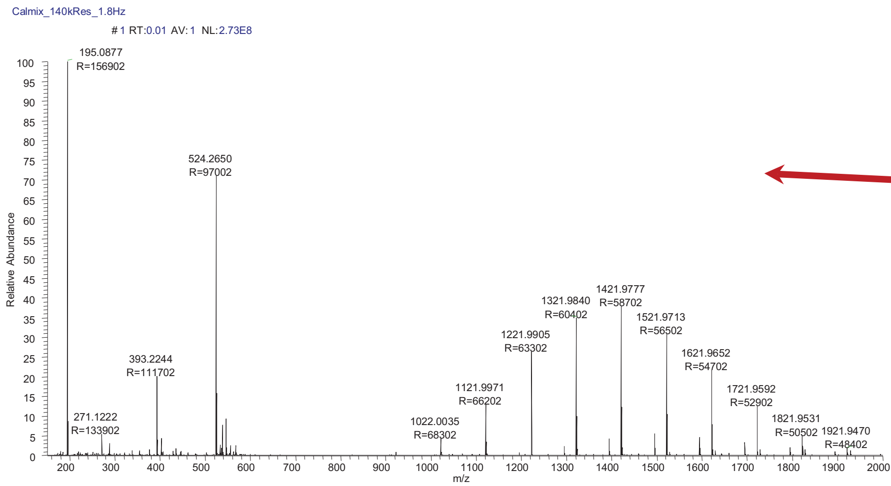
- Quadrupole mass filter
- Enhanced Fourier transform (eFT™) for Orbitrap data processing
- Predictive automatic gain control (pAGC) and parallel filling & detection
- Possibility of multiple fills for spectrum multiplexing
- S-lens for higher transmission (like in LTQ Orbitrap Velos) with rugged optics
- C-trap directly interfaced to HCD (like in LTQ Orbitrap Velos)

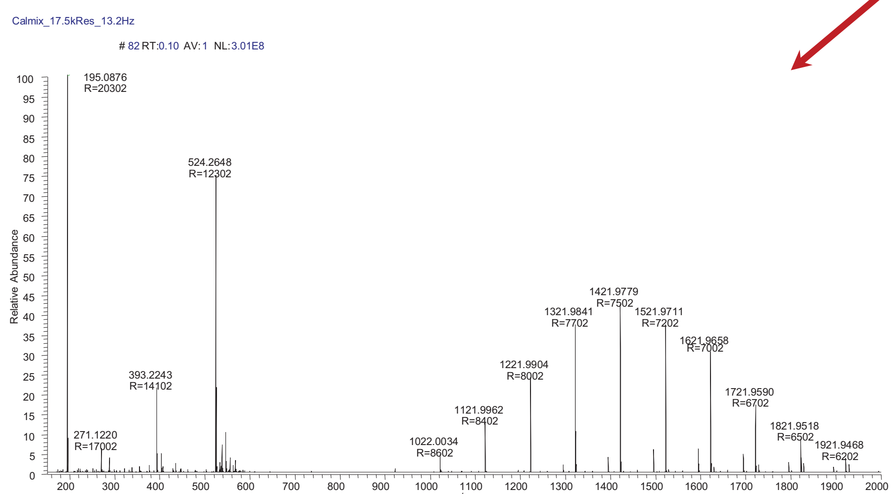


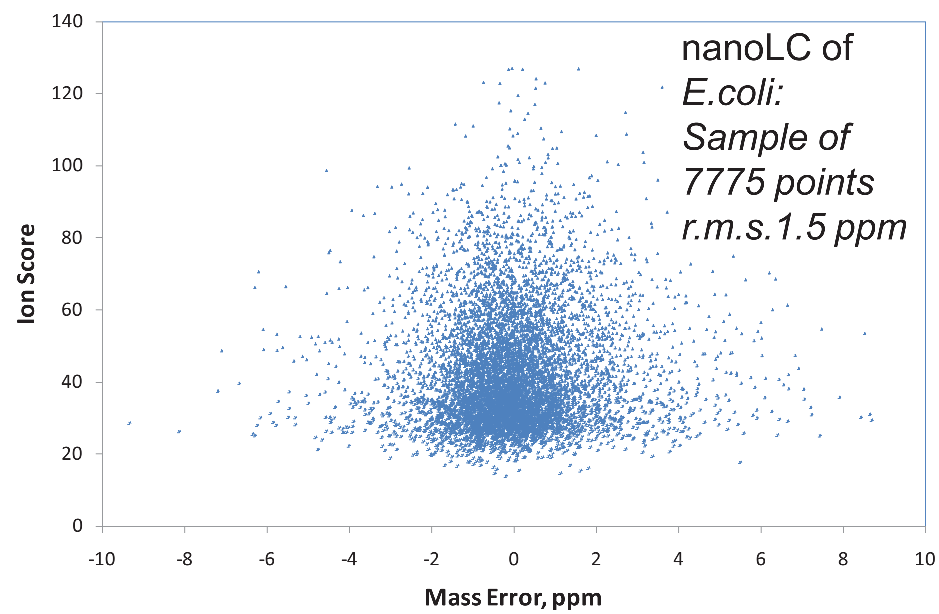
Michalski, A; Damoc, E; Hauschild, JP; Lange, O; Wieghaus, A; Makarov, A; Nagaraj, N; Cox, J; Mann, M; Horning, S "Mass Spectrometry-based Proteomics Using Q Exactive, a High-performance Benchtop Quadrupole Orbitrap Mass Spectrometer". *Mol. Cell Proteomics* **10**, (2011)

Resolving power and mass accuracy of the Orbitrap analyzer

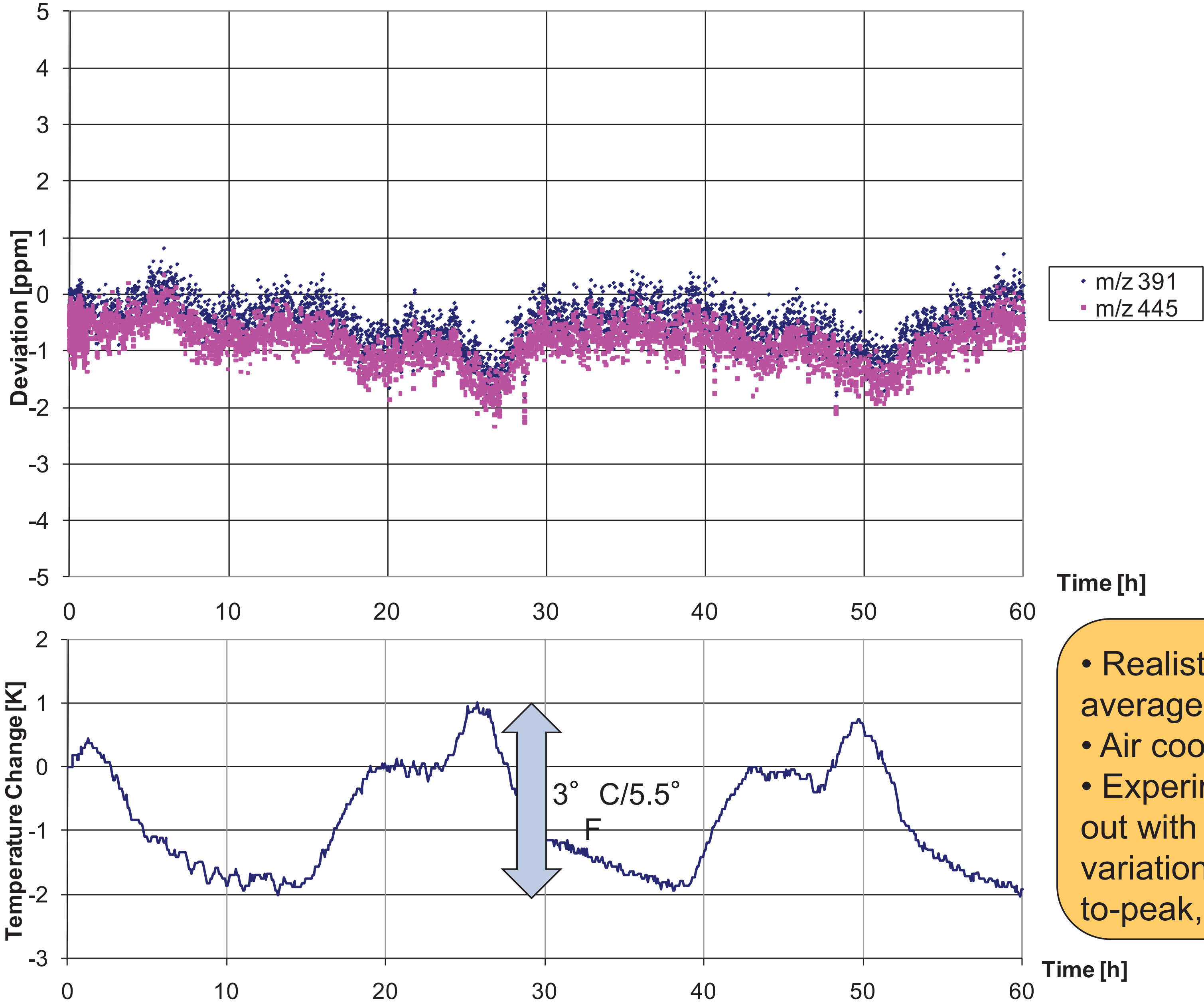
Res setting @ m/z 200	Transient length, ms	Max. scan speed, Hz
140,000	512	1.5
70,000	256	3
35,000	128	7
17,500	64	12





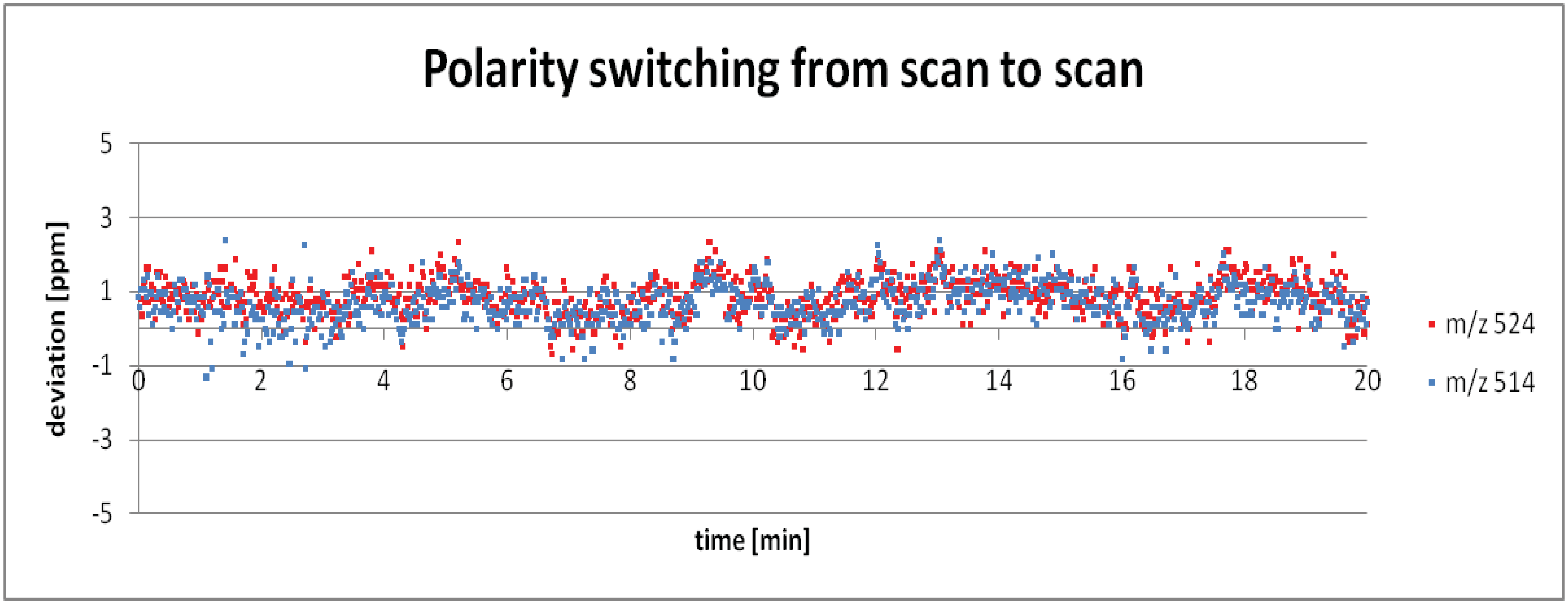


Long-term mass accuracy with external calibration



- Realistic conditions of an average lab
- Air cooling only!
- Experiment was carried out with temperature variations up to 3°C peak-to-peak, up to 1°C/hour

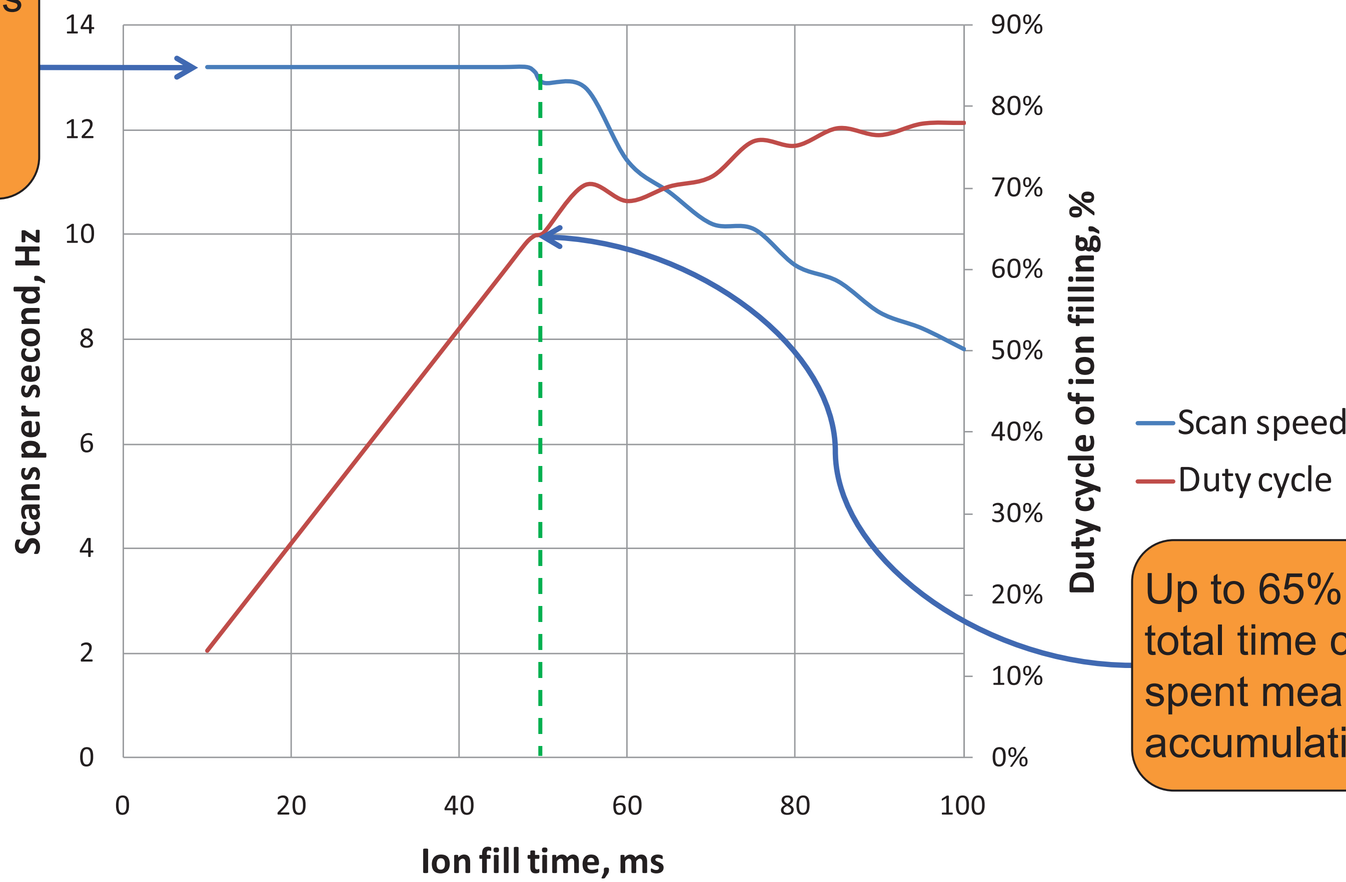
Mass accuracy with polarity switching and external calibration



1 positive + 1 negative scan in 1 second

Spectral acquisition speed with predictive AGC and parallel filling/detection

Scan speed does not change until ion fill time reaches 50 ms

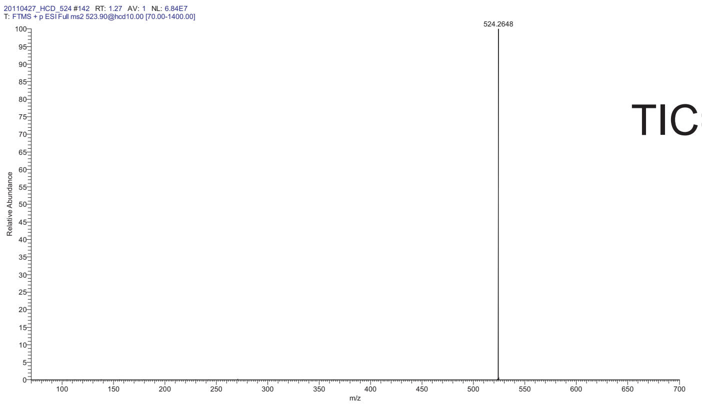


Up to 65% of the total time could be spent meanwhile on accumulating ions!

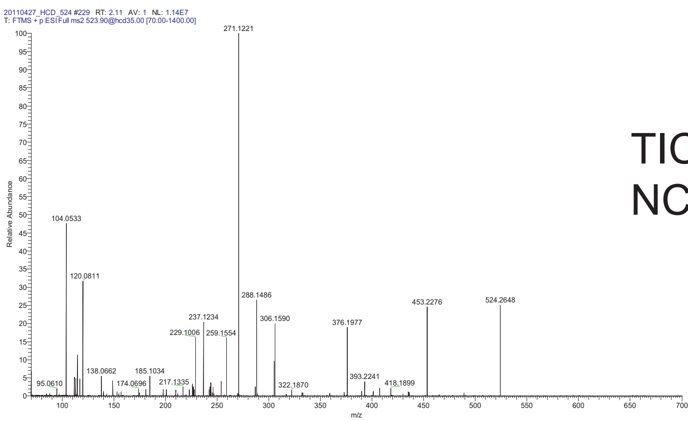
Resolving power setting: 17,500 (min.)
At higher resolving powers, ion fill times and duty cycles are even longer!

HCD performance

m/z 524 (MRFA peptide)

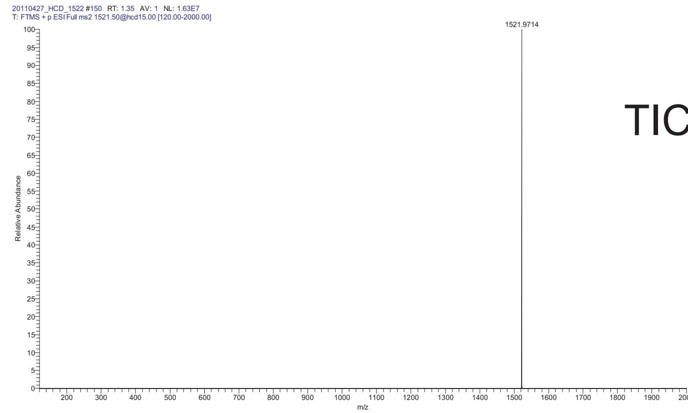


TIC=8.3e7

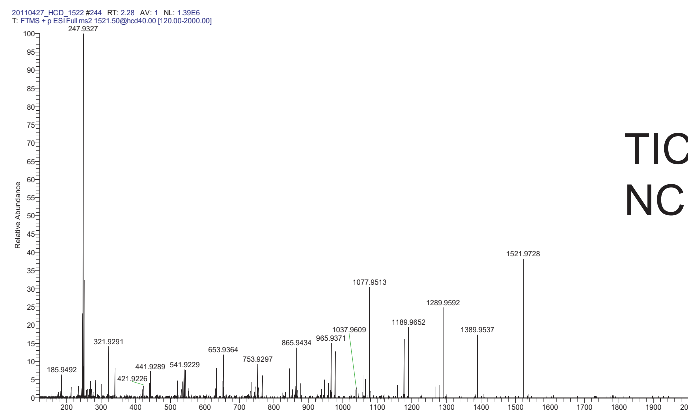


TIC=6.7e7
NCE=35

m/z 1522 (Ultramark)

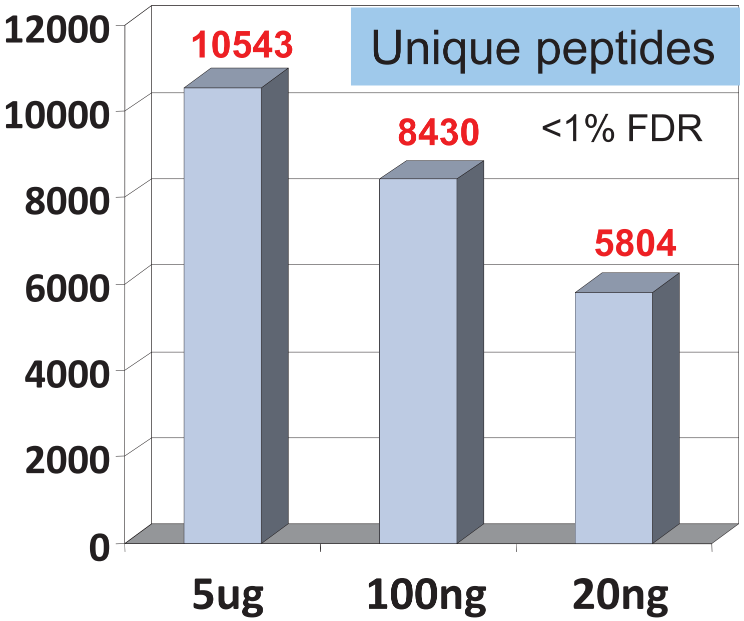


TIC=1.9e7



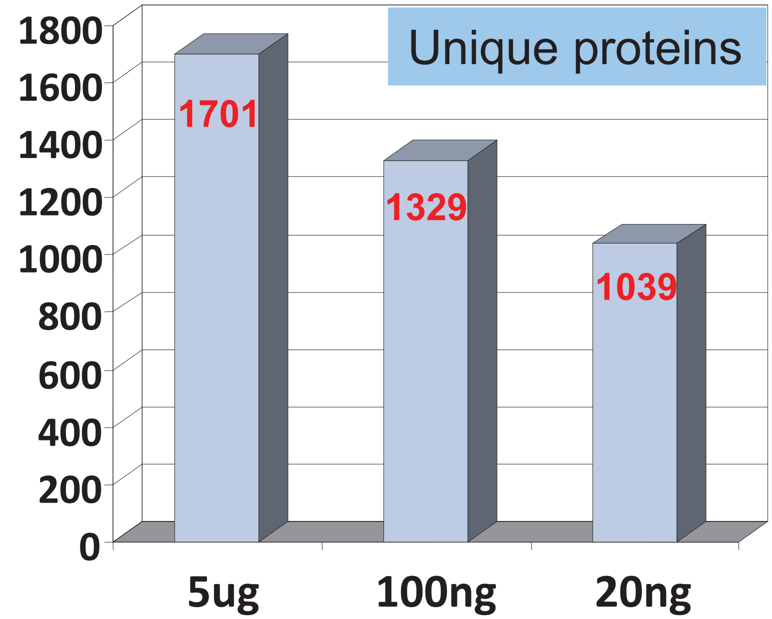
TIC=1.3e7
NCE=40

- Fill times are similar to those in LTQ Orbitrap Velos instrument
- Direct interfacing of the C-trap to HCD cell minimizes losses during fragmentation

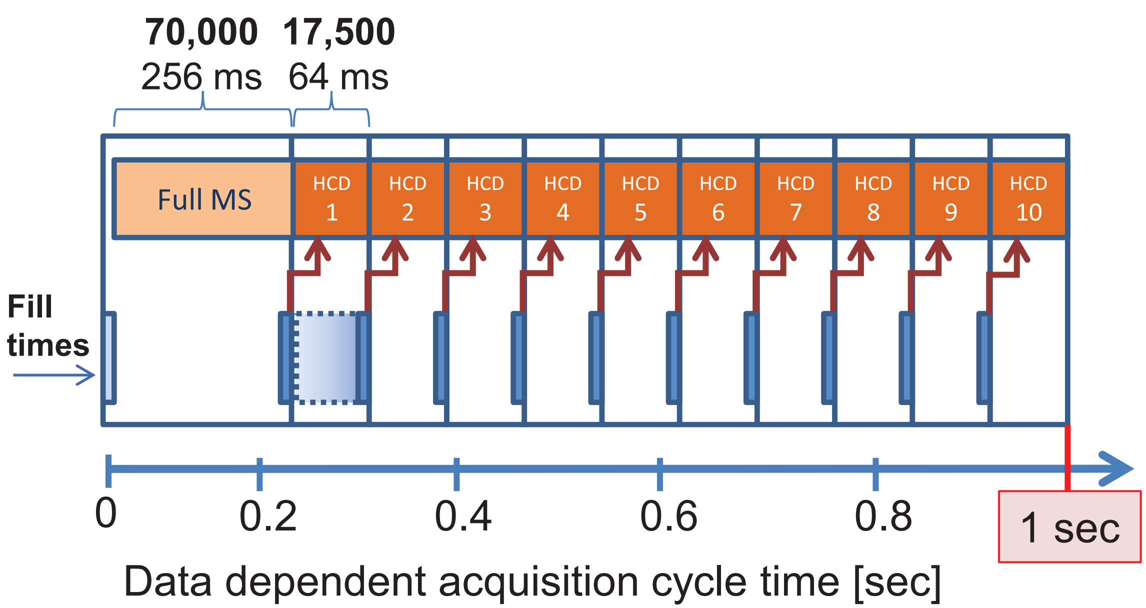


E. Coli digest

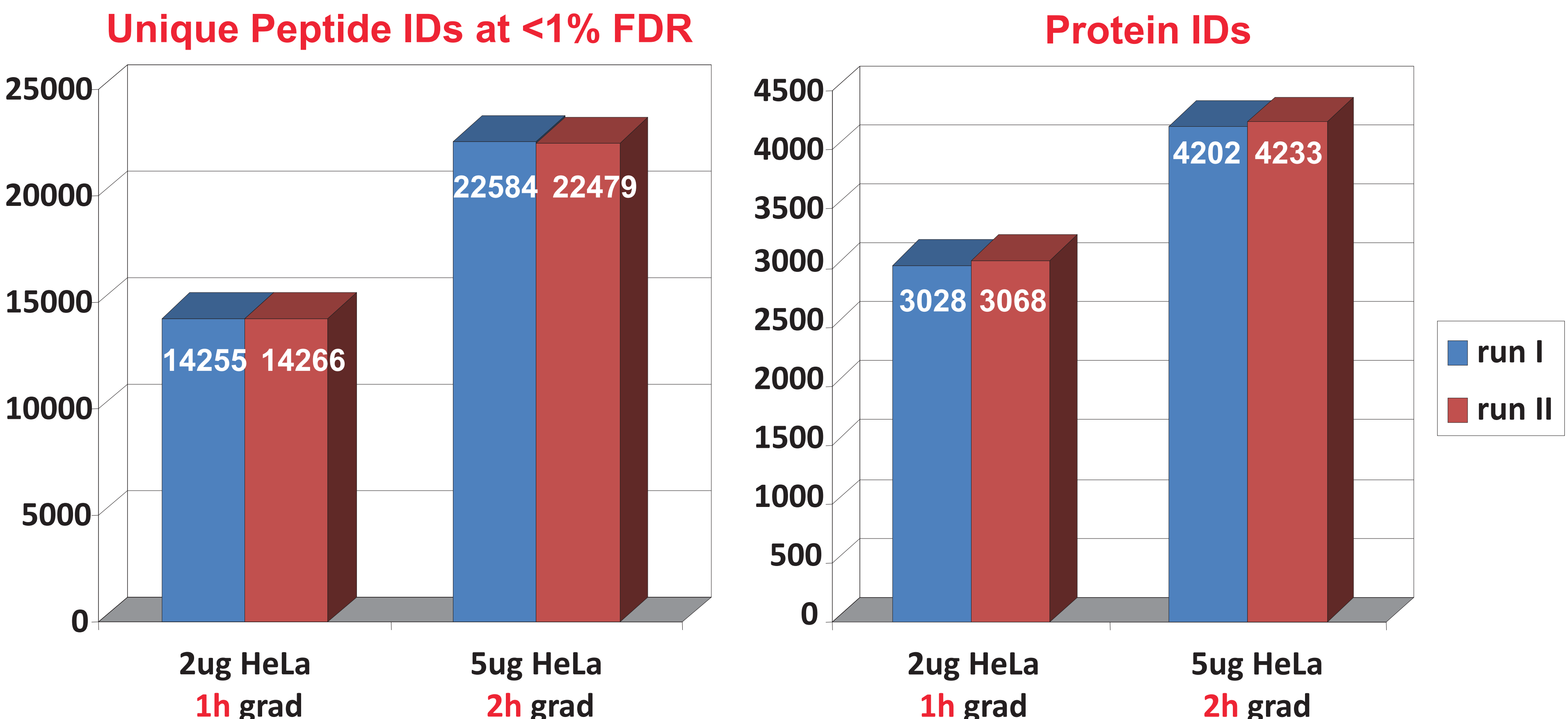
2h gradient, Proxeon NanoUPLC 3 search engines



ddTOP10 HCD acquisition method



Q Exactive LC-MS/MS in action: *HeLa* digest



Total: 17142 27324 3344 4621

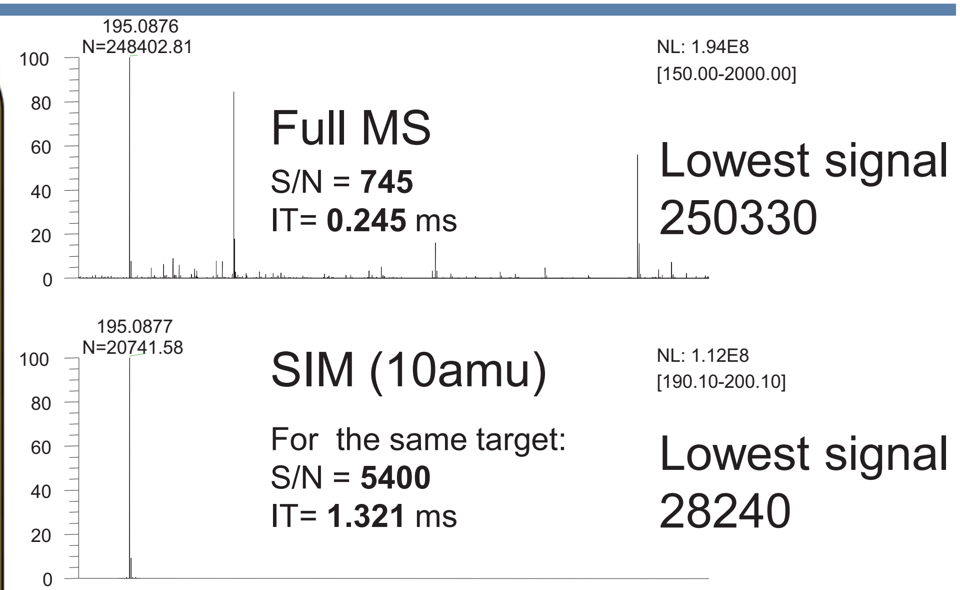
>3000 pph

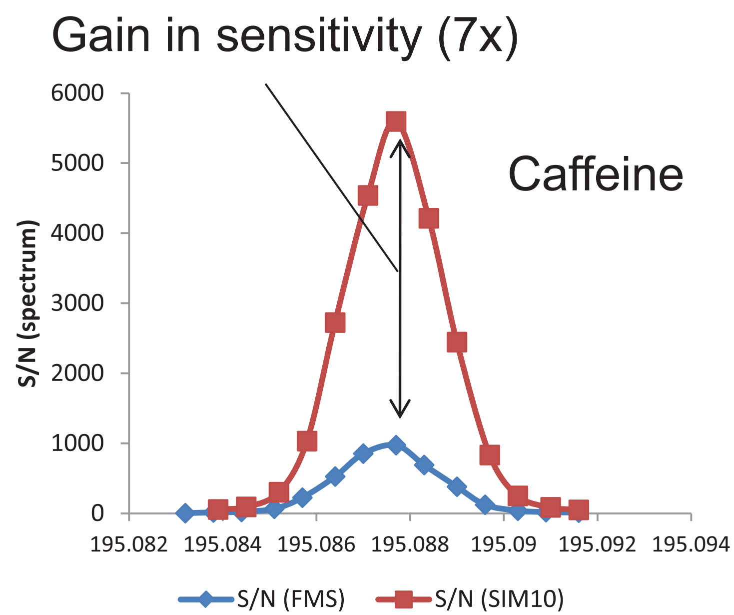
Sample courtesy K. Mechtler

Please see also: C. Paschke; Y. Xuan; E. Damoc; T. Ueckert; U. Comberg; H. Grensemann; M. Kellmann; B. Delanghe. "Breaking the 2000 proteins barrier in a standard LC run using a new benchtop Orbitrap instrument and multiple search engines.". Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011.

What do we gain by selected ion monitoring?

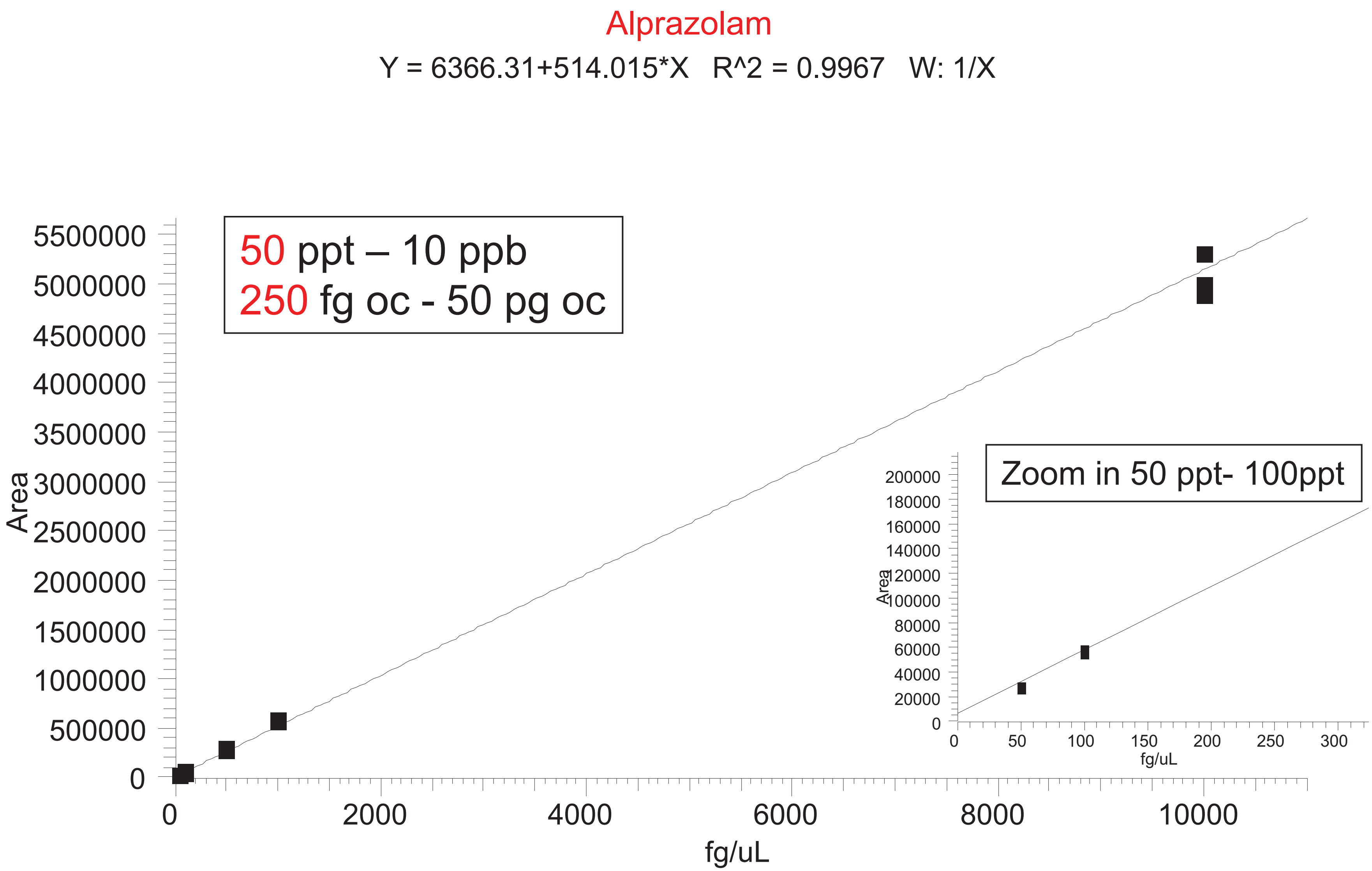
- In Full MS, total C-trap charge capacity is shared between multiple signals of different intensity
- Signal-to-noise ratio becomes dependent on the ratio of compound of interest to other analytes- much less so in SIM!
- In Orbitrap instruments, SIM could become MRM without any additional overhead!



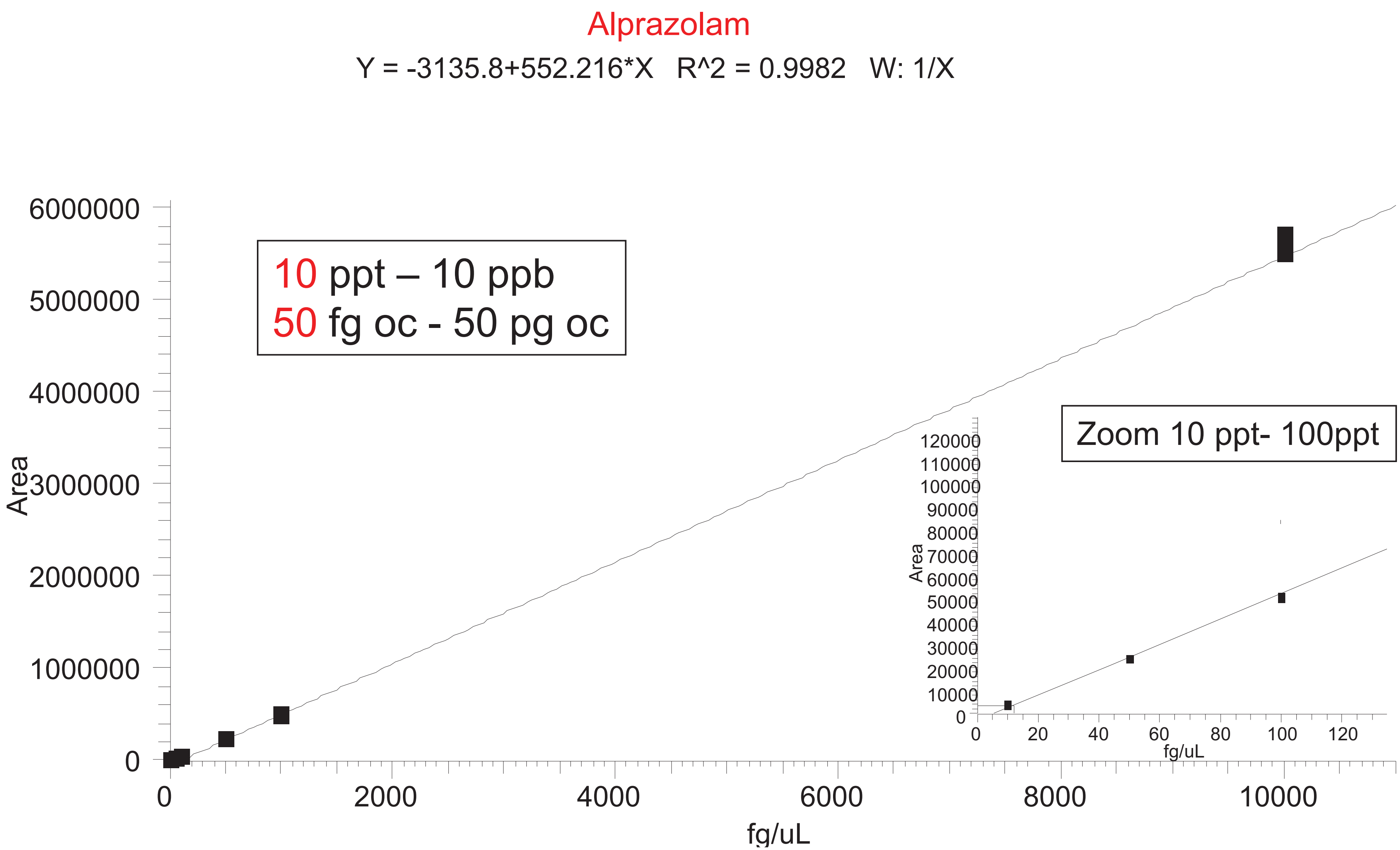


- Sensitivity gain 5 – 10 x with SIM mode
- The gain will be higher in more complex matrices

Alprazolam, Full-Scan Experiment



Alprazolam SIM Experiment



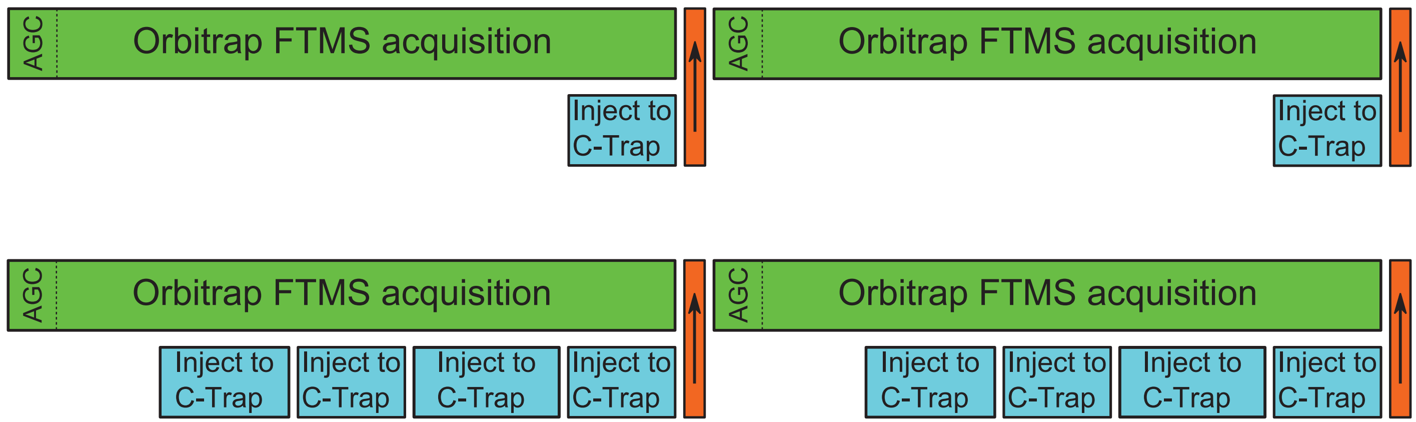
See also: X. He; M. Kozak. "Evaluation of quantitative performance for testosterone analysis in plasma on a novel quadrupole Orbitrap mass spectrometer". Proc. 59th Conf. Amer. Soc. Mass Spectrom., Denver June 5-9, 2011, **Poster WP077**.

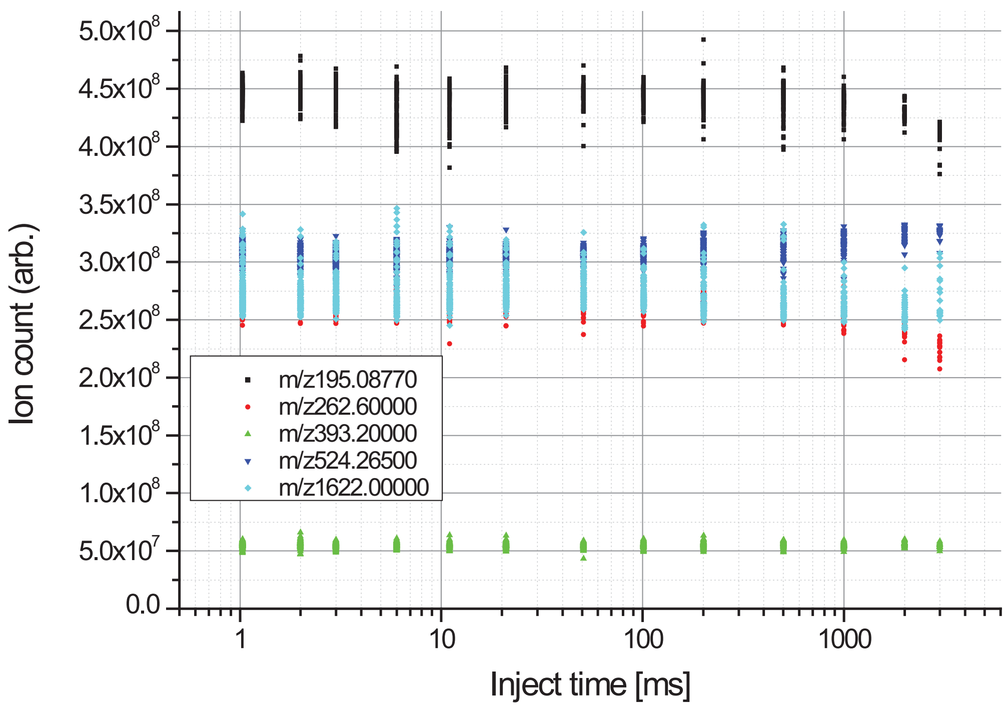
Spectrum multiplexing: principle of operation

Standard operation mode

vs

Spectrum multiplexing





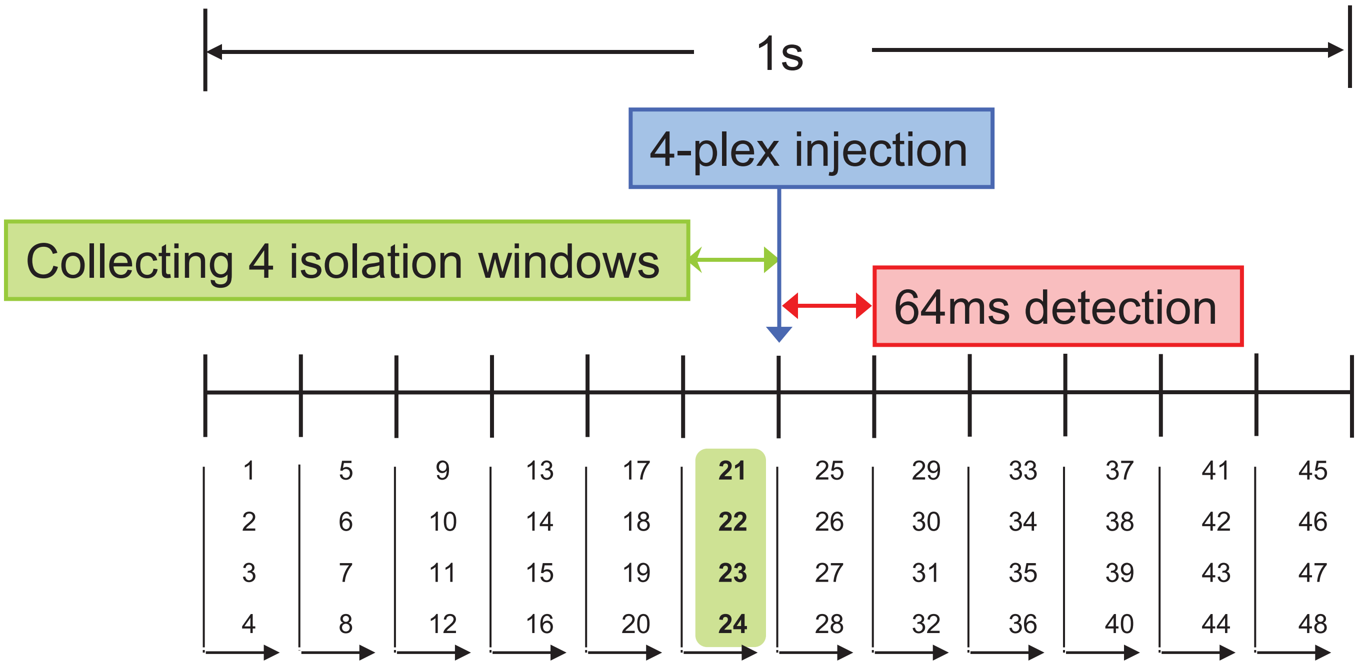
C-Trap storage: No ion loss over a broad range of storage times!

(St.Steel, fused silica, ceramics)

Spectrum multiplexing: example of 4-plex SIM

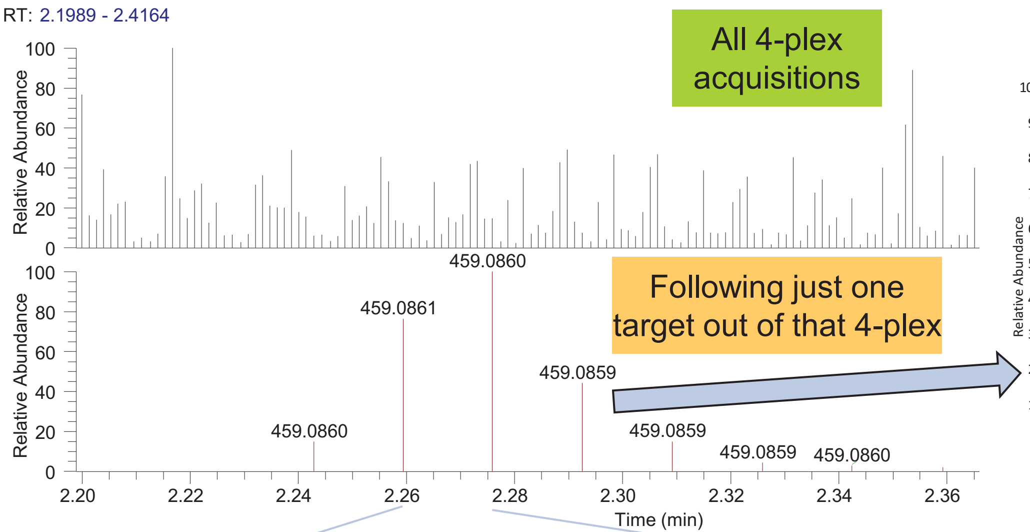
Experimental Setup: 1 Cycle

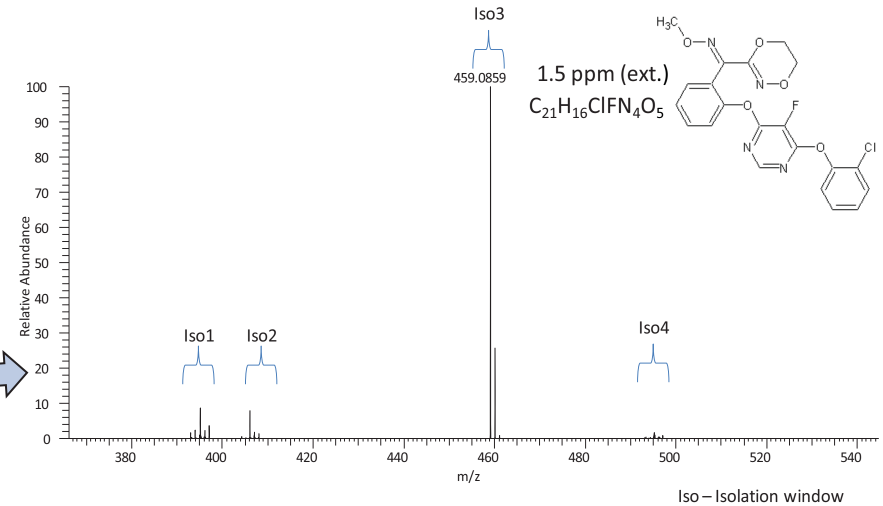
12 Hz Acquisition Rate
48 Precursors / second

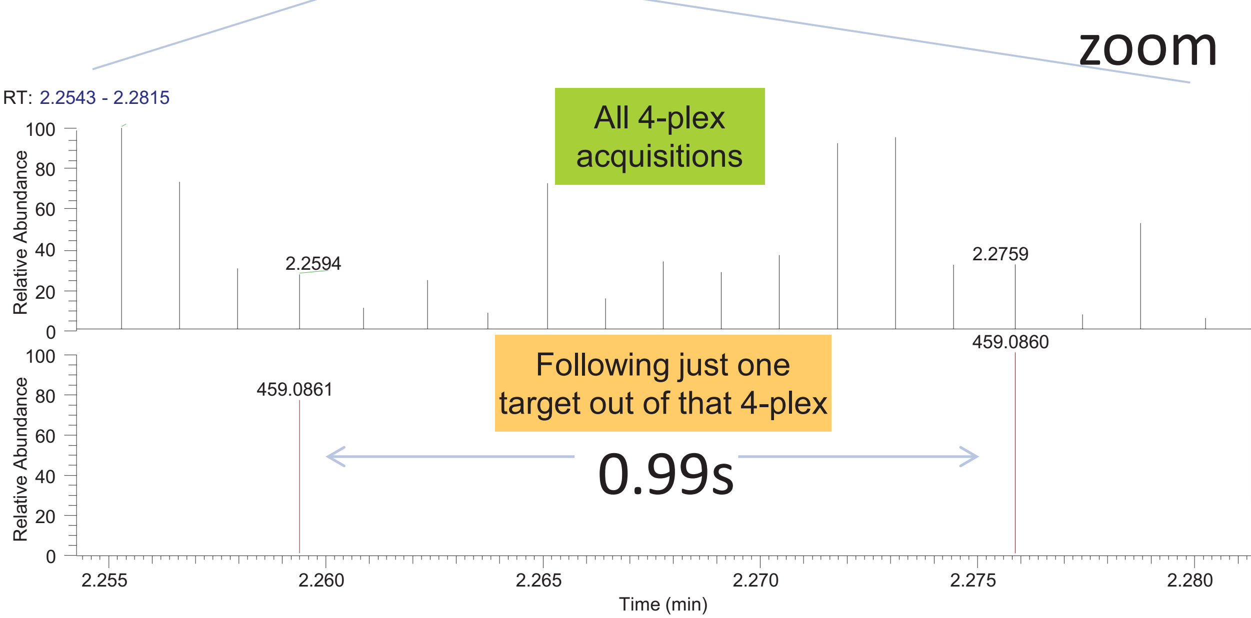


Please see: O. Lange; J.-P. Hauschild; A. Makarov; U. Fröhlich; C. Crone; Y. Xuan; M. Kellmann; A. Wieghaus. “Multiple C-Trap Fills as a Tool for Massive Parallelization of Orbitrap Mass Spectrometry- a new Concept for Targeted Mass Analysis”.

Example Fluoxastrobin, [M+H]+ calc. 459.0866



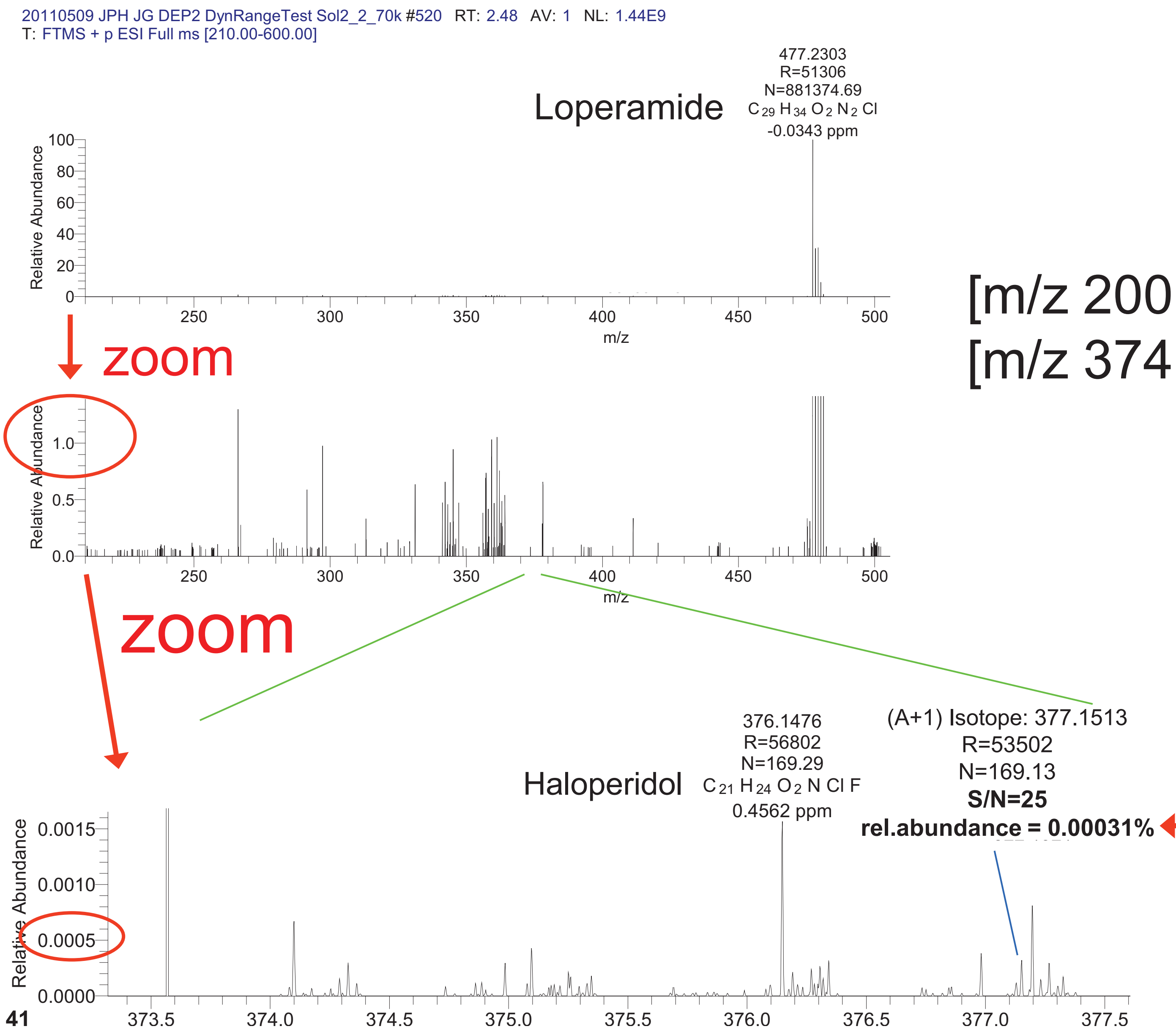




- If we were doing this by conventional SIM, we would have had 4 times less data points across the peak!
- At high resolution and mass accuracy, fragmentation is frequently not needed!

Boosting dynamic range by multiple fills

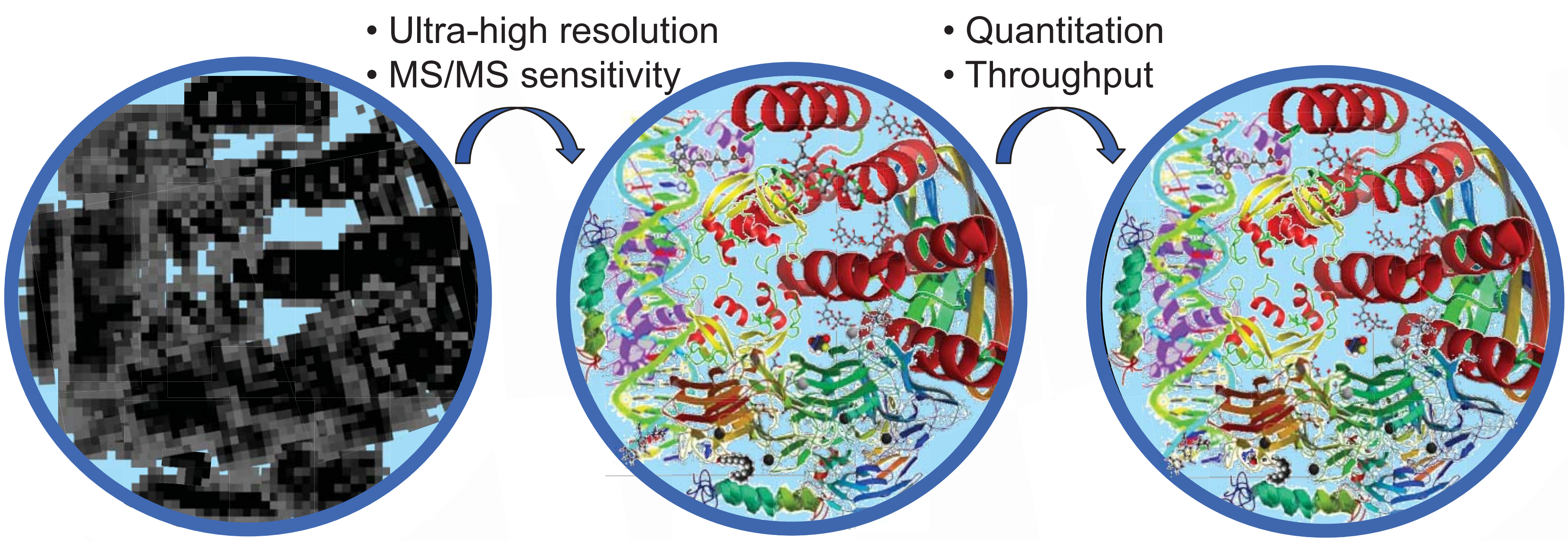
Research only!



[m/z 200..600]@ 0.1ms &
[m/z 374..378]@ 250ms

DR>320.000

Conclusion



- The Orbitrap Mass Analyzer is a new type of mass analyzers with its own unique combination of analytical parameters
- Orbitraps are still evolving…
  - Higher speed
  - Higher resolving power and mass accuracy
  - Higher sensitivity
  - More routine applications
- Exciting new applications continue to emerge

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