Performance Evaluation of a MALDI LTQ Orbitrap XL Imaging Platform Interfaced with a New-Generation Data Acquisition System

Konstantin O. Nagornov,¹ Anton N. Kozhinov,¹ Franklin E. Leach,² Yury O. Tsybin¹

¹ Spectroswiss Inc., Lausanne, Switzerland
² University of Georgia, Athens, USA
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V. Summary
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MALDI Imaging @ 21 T FT-ICR MS

Dynamic range 536 : 1
(6 sigma lower limit)

$R = 875k @ m/z 800$

$f \sim \frac{1}{m/z}$

3.1 s transients

Absorption FT
(aFT) mass spectra
(post-processing)

Figure adapted from:
2020, 3133-3142

Imaging @ FT-ICR MS with high-field magnets is a «gold standard»
MALDI Imaging @ LTQ Orbitrap XL

**Regular resolution, $R$:**
- $60k @ m/z 400: 768 ms$

**Maximum resolution, $R$:**
- $100k @ m/z 400: 1536 ms$

**$f \sim 1/\sqrt{m/z}$ advantage of Orbitrap**
- $42k @ m/z 800: 768 ms$
- $70k @ m/z 800: 1536 ms$

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**aFT:** only via post-processing (AutoPhaser, etc.)

**mFT – magnitude FT (LTQ Orbitrap XL, Velos)**

$R_{aFT} > R_{mFT}$

Higher performance is needed to address challenging applications.
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Approaches to Increase Performance

- Absorption FT (aFT, ~ eFT) to replace the magnitude FT (mFT) processing
  - 2-fold higher resolution compared to mFT, higher mass accuracy
  - Higher sensitivity due to twice shorter (decaying) transient for the same $R$
  - Full profile aFT mass spectra: access to low abundance components
  - Data averaging for user-defined or feature abundant regions of images
  - $SNR$ increases as $\sqrt{N}$ for $N$ transients or full profile aFT mass spectra

- Longer transients acquisition
  - Resolution increases linearly – enabling ultra-high resolution (UHR)
  - Sensitivity increases as a square root (if no decay)

Overall: longer transients and better transients (to enable aFT)
Basics of Absorption FT (aFT): All FTMS Instruments

- Example: 2 signals in a transient
- Same amplitude & frequency
- Different initial phases
  - aFT requires signals from ions of different $m/z$ to have the same initial phases (zero angle).
  
- But: at the time when ion detection starts, individual signals from ions of different $m/z$ have different initial phases, in general.
  
- Post-acquisition phase correction allows setting the same value of initial phase (zero) for the ion signals from different $m/z$.

Fourier transform and absorption mode FTMS:
There exists time $T_0$ prior to $T_1$, when the ions of different \textit{m/z} have (nearly) the same phase.

Ions are ejected from the C-trap (a pulse event) towards the orbitrap.

Ions reach the orbitrap and start oscillations at different times for different \textit{m/z} values (a time-of-flight effect).

At a time $T_1$ when all \textit{m/z} are eventually in the orbitrap, different \textit{m/z} have different phases.

There exists time $T_0$ prior to $T_1$, when the ions of different \textit{m/z} have (nearly) the same phase.

Enabling a\textit{FT} in FTMS: phased transients generation (all phases $= 0$)
Phased Transients: Initial Phases All Close to Zero

Un-phased transients (state of the art)

Phased transients (data in this work)

Mass spectra computed as:
- FT real component (aFT)
- FT magnitude (mFT)

To be an aFT spectrum, this spectrum needs phase correction

Phases equal to zeros: peaks look up. This spectrum is already an aFT spectrum

Phased Transients: Initial Phases All Close to Zero

Comparison of phase distributions (initial phase vs. $m/z$), an example

How to obtain phased transients on LTQ Orbitrap XL?
Upgrade: External Data Acquisition (DAQ) System

MALDI/ESI Source | LTQ | C-Trap


Nagornov, et al., JASMS 2020, 257

Kooijman, et al., Scientific Reports, 2019, 9:8

FTMS Booster enables phased transient generation for FTMS
How to Acquire Longer Transients on LTQ OT XL?

New-generation DAQ system: detection period (# of datapoints to acquire) does NOT have to be preset prior to acquisition or be equal to a power of 2.

External DAQ system

up to 5 s transients were acquired
Upgraded Imaging Platform @ UGA

- LTQ Orbitrap XL
- MALDI/ESI ion source
- Data acquisition system
- Data processing tools
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2-Fold Higher Resolution in the Whole m/z Range

MALDI LTQ Orbitrap XL, 60k @ m/z 400, mouse brain, horizontal section at 12 μm, DHB matrix.

- RAW, mFT, $T_{acq}=768$ ms
- H5, mFT, $T_{acq}=800$ ms
- H5, aFT (half), $T_{acq}=800$ ms

mFT: magnitude FT mode
aFT: absorption FT mode
H5: external DAQ data

Annotated spectra with labels:
- R = 60k @ 400 m/z
- R = 60k @ 400 m/z
- R = 120k @ 400 m/z
- R = 50k @ 800 m/z
- R = 50k @ 800 m/z
- R = 100k @ 800 m/z
- R = 35k @ 1500 m/z
- R = 35k @ 1500 m/z
- R = 70k @ 1500 m/z
Improved Resolution: Lipid Annotation

MALDI LTQ Orbitrap XL 60k @ m/z 400 brain sample

1 – $^{12}$C, $[C_{47}H_{93}N_2O_6P + K]^+$

2 – $^{13}$C$_3$, $[C_{46}H_{90}NO_{10}P + H]^+$

- RAW, mFT, $T_{acq} = 786$ ms
- H5, aFT, $T_{acq} = 800$ ms

averaged mass spectra (22600 scans)

$\Delta m = 17 \text{ Da}$
Improved Mass Accuracy: All Pixels Averaged Data

MALDI LTQ Orbitrap XL, brain sample, 60 k @ m/z 400
33112 pixels, RAW vs H5, averaged mass spectra (22600 scans)

RAW, mFT, $T_{\text{acq}}=768$ ms
Mean: -0.15 ppm
SD: 0.53 ppm

H5, aFT, $T_{\text{acq}}=800$ ms
Mean: 0.03 ppm
SD: 0.27 ppm

Reference masses for internal calibration:

Recalibration method:
Kozhinov et al. Anal. Chem. 2013, 86, 1, 6437-6445

These are mass accuracies of reference compounds
Improved Mass Accuracy: Individual Pixels

RAW, mFT
$T_{acq} = 768$ ms

Mean: -0.41 ppm
SD: 0.49 ppm

H5, aFT
$T_{acq} = 800$ ms

Mean: -0.03 ppm
SD: 0.33 ppm

These are mass accuracies of a compound of interest

Reference masses for internal calibration:

Recalibration method:
Kozhinov et al. Anal. Chem. 2013, 86, 1, 6437-6445
Improved Mass Accuracy: Annotation

single scan #14781
image quality: ± 5 ppm

\[ \Delta m = 9 \text{ mDa} \]

RAW, mFT, \( T_{\text{acq}} = 768 \text{ ms} \)

1 - 13C\textsubscript{2} PC(34:1), C\textsubscript{42}H\textsubscript{82}NO\textsubscript{8}P, [M+H]\textsuperscript{+}

2 - 12C, PC(34:0), C\textsubscript{42}H\textsubscript{84}NO\textsubscript{8}P, [M+H]\textsuperscript{+}

H5, aFT, \( T_{\text{acq}} = 800 \text{ ms} \)

Mean: -3.96 ppm
SD: 0.79 ppm

Mean: 2.24 ppm
SD: 1.27 ppm

Mean: -0.37 ppm
SD: 0.66 ppm

Mean: -0.21 ppm
SD: 0.44 ppm

\[ \Delta m = 9 \text{ mDa} \]

m/z: 762.5916603
m/z: 762.6007369
m/z: 762.5916603
m/z: 762.6007369
High (Image) Dynamic Range

$^{12}$C, $[C_{42}H_{82}NO_8P + K]^+$

$^{12}$C, $[C_{44}H_{86}NO_{10}P + H]^+$

MALDI LTQ Orbitrap XL brain sample, 60k @ m/z 400

Spectral dynamic range: ratio of the most abundant to the least abundant detected ion signals (or to noise level)

H5, aFT, $T_{acq} = 800$ ms

DR ~ 3.0 orders
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>4-fold Higher Resolution in the Whole $m/z$ Range

MALDI LTQ Orbitrap XL, brain sample, 100 k @ $m/z$ 400, **RAW** vs **H5** (DAQ), scan #10731

- RAW, mFT, $T_{acq}=1.5$ s
- H5, mFT, $T_{acq}=3.5$ s
- H5, aFT (half), $T_{acq}=3.5$ s
Improved Resolution: Lipid Annotation

MALDI LTQ Orbitrap XL
100 k @ m/z 400
Brain sample
Image quality: ± 3 ppm

- RAW, mFT, $T_{acq}=1.5$ s
- H5, aFT, $T_{acq}=3.5$ s

averaged mass spectra (6540 scans)

$\Delta m = 9 \text{ mDa}$
Improved Resolution: Lipid Annotation

MALDI
LTQ Orbitrap XL
100k @ m/z 400
Brain sample
Quality: ± 3 ppm

1 $^{13}$C$_2$, SM(d42:2) C$_{47}$H$_{93}$N$_2$O$_6$P, [M+K]$^+$
2 $^{12}$C, SM(d42:1) C$_{47}$H$_{95}$N$_2$O$_6$P, [M+K]$^+$

- RAW, mFT, $T_{acq}$ = 1.5 s
- H5, aFT, $T_{acq}$ = 3.5 s
averaged mass spectra (6540 scans)

$\Delta m = 11$ mDa
Improved Sensitivity for Resolved Peaks

MALDI LTQ Orbitrap XL
100 k @ m/z 400
Brain sample
Quality: ± 3 ppm

Weak signal @
m/z 849.64089

Strong signal @
m/z 849.62456

Δm = 16 mDa

Improved performance helps to reveal boundaries between tissue regions, etc.
High (Spectral) Dynamic Range @ UHR

MALDI Orbitrap XL
15_brain_100k Scan #7354

Dynamic Range = 625 : 1

SM(d16:1/18:0) [C_{39}H_{79}N_{2}O_{6}P + K]^+

PC(32:0) [C_{40}H_{80}NO_{8}P + K]^+

8.5 mDa

R = 480k
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The Figures of Merit: MALDI LTQ Orbitrap XL

<table>
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<th>Performance</th>
<th>Before Upgrade (RAW)</th>
<th>After Upgrade (H5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution @ m/z 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>regular</td>
<td>60k</td>
<td>120k</td>
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<tr>
<td>max</td>
<td>100k</td>
<td>500k</td>
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<tr>
<td>Mass accuracy</td>
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<tr>
<td>regular</td>
<td>1.8 ppm</td>
<td>1.2 ppm</td>
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<tr>
<td>max</td>
<td>1.4 ppm</td>
<td>1.0 ppm</td>
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<tr>
<td>Dynamic range (image or spectral)</td>
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</tr>
<tr>
<td>regular</td>
<td>2.6 orders</td>
<td>3.0 orders</td>
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<td>max</td>
<td>2.7 orders</td>
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<tr>
<td>Speed (scans per second)</td>
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<tr>
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<tr>
<td>Annotations (FDR 10 %)</td>
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<td>114</td>
</tr>
<tr>
<td>max</td>
<td>37</td>
<td>52</td>
</tr>
</tbody>
</table>
Conclusions

A cost-efficient imaging platform that integrates:
- ESI mass spectrometer (LTQ Orbitrap XL)
- Imaging-grade MALDI/ESI source (Spectroglyph)
- Advanced data acquisition system (FTMS Booster)
- Advanced data processing software (Peak-by-Peak)

Improved performance supports discoveries of new biological insights:
- High to ultra-high resolution in the whole $m/z$ range
- High mass accuracy, sensitivity, dynamic range and speed

Applicability limits of the approach:
- MALDI-related scan-to-scan variations of the total injected charge (phase validation / fine tuning to be enabled in FT processing).
- Achievable levels of analytical characteristics likely vary with the quality of a particular Orbitrap system available for the researcher.
Thank you!

Jon Amster

Mikhail Belov

Alexandrov team