The Power of MS\textsuperscript{n} Combined with the Simplicity of MALDI
The addition of MALDI (Matrix-Assisted Laser Desorption/Ionization) to the Thermo Scientific LTQ XL linear ion trap produces information-rich spectra that are essential for the analysis of digested proteins, peptides and post-translational modifications (PTMs). The tissue-imaging solution provides researchers with unparalleled sensitivity and unmatched MS spectral quality of linear ion trap technology as well as faster sample analysis, compared with the standard practice of homogenizing and extracting from tissue. For small molecule applications, the MALDI LTQ XL with Thermo Scientific ImageQuest software offers pathologists and pharmaceutical researchers a complete package for the acquisition and presentation of MS images directly from tissue samples.
Thermo Scientific MALDI LTQ Orbitrap™ series simplifies the analysis of whole tissue, biological and polymer samples without extensive sample preparation. The new MALDI source, available on the Thermo Scientific LTQ Orbitrap XL and LTQ Orbitrap Discovery, is ideally suited for proteomic and metabolism applications. For the first time, resolutions of greater than 50,000 FWHM and mass accuracies of 1-2 ppm are routinely available for MS, MS/MS and even MS^n data.

The Thermo Scientific LTQ Orbitrap received the R&D 100 Award in 2006 and the Pittcon Editors’ Gold Award at Pittcon 2006.
The Power of MS\textsuperscript{n} Combined with MALDI LTQ XL and MALDI LTQ Orbitrap

The Thermo Scientific MALDI LTQ XL consists of a highly sensitive linear ion trap with fast sample analysis. This provides more reliable and definitive answers for protein identification and structural elucidation through MS\textsuperscript{n}. The MALDI LTQ Orbitrap series provides fast, reliable, and confident protein identification and enables additional analytical approaches to localize post-translational modifications. Outstanding mass accuracy and high sensitivity make the MALDI LTQ Orbitrap a superior alternative to traditional MALDI-TOF systems.

**MALDI LTQ XL AND MALDI LTQ ORBITRAP OFFER:**
- High throughput analysis of in-gel digests for protein ID
- CID, PQD, and HCD for complementary fragmentation information
- Direct tissue analysis by MALDI Imaging
- ImageQuest software for visualization of tissue imaging data

**Imaging: Peptide Localization in MALDI Thin Layer Preparation**

FTMS + c MALDI Full ms [130.00 - 1500.00]

**MALDI with Orbitrap Detection: Mass Accuracy and Resolving Power**

Mellitin @ RP 50,000, \( \leq 1 \) ppm mass accuracy. Sequence of Mellitin from honey bee: GIGAVLKVLTTGGLPALSWIKRKRQQ

Sum Formula: C\textsubscript{131}H\textsubscript{230}N\textsubscript{39}O\textsubscript{31}; Monoisotopic Mass MH+: 2845.7614; N-Terminus: H; C-Terminus: Amidation
**Peptide Mass Fingerprint:**
High Protein Coverage @ Low Digest Loads

FTMS + p MALDI Full ms [500.00-4000.00]

PMF, Peptide Mass Fingerprints were obtained from a 5 fmol load of an enzymatic digest of Bovine Serum Albumin, 4-hydroxy-alpha-cyano cinnamic acid (HCCA) matrix. Five scans, total of 100 laser shots, RP 30,000 @ m/z 400, stainless steel sample plate. The insets show some of the observed proteolytic peptides. Monoisotopic MH+ masses are submitted to MASCOT™ for protein identification. Protein is unambiguously identified as Bovine Serum Albumin, sequence coverage is 50 %. C@ represents the carboxymethylated Cysteine.
MALDI and Tissue Imaging with

The Thermo Scientific MALDI source coupled with ImageQuest software is an advanced data visualization software designed for tissue imaging. Samples can be prepared and analyzed without the need to extract them first. ImageQuest provides intuitive and powerful software to pathologists and pharmaceutical researchers for the presentation of MS images directly from tissue samples.

MALDI LTQ XL AND IMAGEQUEST OFFER:
- Ability to overlap distribution of two or more analytes in one picture
- Three interlinked images for all aspects of tissue imaging with MALDI
- Threshold to eliminate undesired matrix ions
- Visualization with user-definable intensity axis and linear or logarithmic scaling
- 3-D image can be rotated for complete visualization

Step 1: Section tissue
Thermo Scientific FE/FSE Cryotome

Step 2: Apply MALDI matrix

Step 3: Transfer tissue samples into MALDI LTQ XL for MS, MS^n
Localization of Cancer Drug in a Human Tumor

MS² of the drug metabolite in complex biological tissue samples will most likely isolate isobaric interferences, such as phospholipid signal (m/z 184), and poor signal-to-noise for the compound of interest (m/z 393, top trace). Obtain specific isolation of the metabolite with MS³, which yields expected fragment ion (m/z 349, bottom trace) that can be easily mapped to display metabolite localization within the tumor (2-dimensional image on the left).

Step 4: Visualize MALDI data using ImageQuest 1.0
The new MALDI source, available on the LTQ Orbitrap XL and the LTQ Orbitrap Discovery, is ideally suited for proteomics and small molecules applications. Applications include the high-throughput analysis of in-gel digests of 2D gel spots, de novo sequencing, TMT®, and iTRAQ™ quantitation, tissue imaging, and small molecule analysis.

**THE MALDI LTQ ORBITRAP OFFERS:**
- High mass resolution combined with accurate mass measurement in MS and MS+n mode for MALDI-produced ions
- Information-rich MSⁿ spectra, confident structural elucidation
- Long lasting, reliable mass calibration
- No need for internal standards or daily recalibration
- Intuitive instrument control and data processing software

**Protein ID upon MS/MS**

Data obtained from an LC MALDI acquisition of an enzymatic digest of Purple Membrane. Data show MS full scan and MS/MS data of a given retention time. Higher Energy Collisional Dissociation (HCD) upon the given MS full scan is used to identify the peptide @ m/z 2163. The peptide with the sequence SRAFGEAEAPPSAGDGAATS derives from Bac. Rhodopsin (Purple Membrane).

**Low Attomol Peptide Loads**

50 attomol (0.5 µL) total peptide load of Angiotensin II applying a focusing step preparation, Focus Chip, Qiagen®, 4-hydroxy-alpha-cyano cinnamic acid matrix. Only 40 laser shots (= 1 e⁵ charges) are needed for the single scan shown. Sample consumption per scan is estimated to sub attomol.

**Angiotensin II**

+1.12 ppm

**FTMS + p MALDI Full ms [820.00-2000.00]**
**LDI LTQ Orbitrap Discovery**

### Small Molecules

Mixture of Atropine, Sulfadimethoxine, Buspirone, and Reserpine; 4-hydroxy-alpha-cyano cinnamic acid matrix, each compound is 500 fg on plate.

Reserpine @ m/z 609 and its unsaturated relative @ m/z 607 can be distinguished in a mixture not only in MS by accurate mass measurement but also by MS/MS.

### Narrow Isolation Widths for the MS/MS Experiments

An isolation width of 1.5 u was chosen for each of the compounds. Spectra were obtained by Collision Induced Dissociation (CID) in the linear ion trap followed by Orbitrap detection for the 500 fg load. Narrow isolation width allows to substantially reduce chemical noise and significantly improves library searches.

### Table

<table>
<thead>
<tr>
<th>m/z</th>
<th>Theo. Mass</th>
<th>Δ (ppm)</th>
<th>RDB e-</th>
<th>Composition</th>
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<tr>
<td>290.17542</td>
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<td>6.5</td>
<td>C₃₂H₂₄O₃N₂ Atropine</td>
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<td>C₂₃H₃₀O₃N₂ Reserpine</td>
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<td>607.26581</td>
<td>607.26501</td>
<td>1.32</td>
<td>15.5</td>
<td>C₂₃H₃₀O₃N₂ Unsaturated by-product of Reserpine</td>
</tr>
</tbody>
</table>

Mass accuracy of full scan information, external mass calibration.
Higher Energy Collisional Dissociation

The LTQ Orbitrap XL and the LTQ Orbitrap Discovery feature a collision cell to provide additional flexibility to any MS/MS experiment. Ions can be selected in the linear ion trap and fragmented either in the ion trap (CID) or in the collision cell (HCD).

MALDI MS/MS @ m/z 1563.73, a Synthetic Peptide of the Sequence REINHOLDPESCH

Sum Formula: C_{64}H_{103}N_{22}O_{22}S; Monoisot. Mass MH^+: 1563.73325, O is Ornithine

Intact Peptide

FTMS + p MALDI Full ms [500.00-2000.00]

MH^+ (theory): 1563.7333

CID, PQD, HCD

MALDI LTQ ORBITRAP IS IDEALLY SUITED FOR DE NOVO SEQUENCING BASED ON:

- Accurate mass measurement of precursor and fragment ions
- Fragment ions obtained by various fragmentation techniques CID, PQD, HCD
- Low energy fragmentation plus immonium ions and internal cleavage information is obtained

Accurate Mass Measurement excludes II/II/II/I

Hypl / Hypl / IHypl / LHypl for $\Delta = 226.0965$

FTMS + p MALDI d w Full ms? 1563.73 @ cld45.00 [420.00-1575.00]

Dipeptides other than PE/EP can be excluded.
For HCD (Higher Energy Collisional Dissociation) ions are passed through the C-trap into the gas-filled collision cell. Normalized Collision Energy in HCD MS/MS experiments provides reproducible data from instrument to instrument.

A synthetic peptide is sequenced using CID and HCD fragmentation techniques followed by Orbitrap detection. Complete b- and y-ion series are observed. These fragments come along with a- ions, internal cleavages and – in the low mass range – a number of immonium and immonium-related ions.
THermo SCIentIFIC SApplICAtion-Specific SOftware
TURNING DATA INTO INFORMATION

Xcalibur™ data system
Stable operating platform
Xcalibur is the versatile, easy-to-use data system that controls all Thermo Scientific MS systems. The Xcalibur home page offers easy navigation through the process of instrument setup, sequence setup, and data acquisition. The XReport package simplifies custom reporting with drag-and-drop functionality.

Protein Calculator
Protein Calculator performs in silico digestion on specified peptide sequences with post-translational modifications. The proteolytic fragment spectra can be saved as RAW data files for comparison with acquired spectra.

Xtract
Xtract deconvolutes isotopically resolved data, simplifying complex MS/MS spectra acquired in top-down, intact protein analysis. Specify the mass range, mass resolution, and S/N criteria for deconvolution and display in one of four modes: monoisotopic masses, isotopic pattern, or approved or disapproved signals. The results can be exported as RAW or ASCII file formats.

Proteome Discoverer
Mass informatics platform for protein scientists
Proteome Discoverer is a new, workflow-based, proteomics data processing software for in depth data mining of complex LC-MS® data sets. With the ability to exploit data from different dissociation techniques (CID, HCD, IRMPD, ETD and ECD), Proteome Discoverer provides extra certainty for peptide and protein identifications. Optional inclusion of multiple search algorithms increases analytical flexibility, and results can now be merged into a single report for easier interpretation.

ImageQuest Software
ImageQuest Software provide tools to visualize small molecule imaging data obtained by MALDI through 2D- and 3D maps of the analyzed tissue

ProSightPC™
ProSightPC was developed to address the specific requirements of a top-down and middle-down proteomics strategy. The fragmentation spectra of multiply-charged, intact proteins are exceedingly complicated. ProSightPC processes high resolution, accurate mass data from the Thermo Scientific LTQ Orbitrap to produce a list of neutral fragment masses and performs comparisons to proteome databases to identify and characterize proteins.