The new autoflex speed

- Increased Efficiency in MALDI-TOF MS
Accelerated Results with Increased Speed and Efficiency

The autoflex™ speed series of MALDI based mass spectrometers has been designed to significantly increase productivity across a broad range of applications. With advances in performance and system capabilities, the autoflex speed series has the power, flexibility, and utility to be deployed in many analytical operations.
The autoflex speed series contains several system options and configurations and can be customized to best suit the applications at hand. The members of the product family include:

**autoflex speed L**
A linear mode system with outstanding dynamic range optimized for high mass range (>15 kDa) applications and MALDI Tissue Imaging.

**autoflex speed LRF**
A reflectron containing system designed to include enhanced applications like peptide analysis; also capable of operation in linear mode.

**autoflex speed TOF/TOF**
A high performance system providing all the capabilities of the autoflex series including MS/MS for detailed sample characterization.

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### Versatility: autoflex speed

<table>
<thead>
<tr>
<th></th>
<th>Mode(s) of Operation</th>
<th>System Speed</th>
<th>Protein TDS</th>
<th>TLC-MALDI</th>
<th>Small Molecule Imaging</th>
<th>Protein Tissue Imaging</th>
<th>High Mass Application, Macromolecules, Polymer Analysis</th>
<th>Proteomics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>autoflex speed L</strong></td>
<td>linear mode</td>
<td>2 kHz</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td><strong>autoflex speed LRF</strong></td>
<td>linear / reflector mode / FAST-SRM</td>
<td>2 kHz</td>
<td>✓</td>
<td>✓</td>
<td>✓ incl. FAST-SRM</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>autoflex speed TOF/TOF</strong></td>
<td>linear / reflector mode / TOF/TOF</td>
<td>2 kHz MS 200 Hz MS/MS</td>
<td>✓ incl. T^1-Sequencing</td>
<td>✓</td>
<td>✓ incl. FAST-SRM or TOF/TOF</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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</table>
Harnessing the Power of Innovation

The autoflex speed family of products encompasses numerous technology advances that provide researchers with:

- Unmatched analytical speed using smartbeam™-II, a 2 kilohertz laser and system electronics for intensive or high throughput applications. The MALDI customized smartbeam-II technology is exclusively developed, manufactured and maintained by Bruker for unsurpassed MALDI results.
- Expanded mass range capabilities to handle intact proteins and molecules such as large polymers.
- Superb broadband mass resolution and mass accuracy through proprietary PAN™ technology.
- Novel FlashDetector™ technology increases resolution and sensitivity and prevents signal saturation for complex samples.
- Minimized maintenance with IR laser-based self-cleaning MALDI Perpetual™ Ion Source, which improves system up-time and utility.
- Optimized software packages for a range of protein, peptide, and polymer analysis workflows.
- Redefined routine and generic applications.

Transforming the challenging into the routine

One of the key benefits of the autoflex speed series is that these systems were designed and optimized to be easy to use, robust systems for both traditional MALDI mass spectrometer applications (protein, peptide, and polymer analysis) as well as cutting edge work (MALDI Imaging, glycan analysis). With this combination of performance and utility, the autoflex series is an excellent choice for a number of application areas including:

- Proteomics research
- Biomarker discovery
- Analysis of biotherapeutics
- Bioassay development and metabolite distribution
- Molecular histology/MALDI Imaging
- Polymer analysis
- Glycan and glycoprotein analysis
- Microorganism identification
- Intact Protein Sequencing

Self-cleaning ion source based on laser-irradiation

MALDI Perpetual Ion Source: The proprietary MALDI Perpetual Ion Source is automatically laser-cleaned in minutes. The unique, laser-based cleaning process removes all MALDI matrices without residues. Extraction plate temperature profile during the cleaning process (A). (B) Extraction plate before and (C) after the laser cleaning process.
Top-Down Protein Sequencing in Minutes

MALDI-TOF has been used for many years as a standard in protein and peptide analysis. The autoflex speed MALDI-TOF/TOF systems extend these capabilities to further reaching protein sequencing known as Top-Down Sequencing (TDS), whereby the N- and C-terminal amino acid sequences of proteins are analyzed directly. Even proteins with blocked N-terminus can be sequenced using MALDI-TDS.

This capability can be extremely useful when analyzing known proteins such as biotherapeutics. MALDI-TDS can, e.g., assign the N-term pyroglutamylation and the C-term Lysine truncation status of the heavy chain of therapeutic antibodies basically in a minute.

**Top-Down Sequencing Analysis of an Intact IgG**

![Image of MALDI-TOF spectrum](image)

Intact MALDI-TOF spectrum of a monoclonal antibody (mAb) in sinapinic acid defines the molecular weight.

MALDI Top-Down Sequencing (TDS) spectrum of the intact mAb provides sequence calls from N- and C-terminus with monoisotopic peak assignment. Here, the N-terminal sequence of the HC is assigned confirming the N-terminus being pyroglutamylated.

T³-Sequencing spectrum of the c²-MALDI-TDS fragment ion was IDed through standard Mascot search. It allowed the extension of the N-terminal readout and the confirmation of the pyroglutamylated N-terminus.

Bruker’s advanced bioinformatics solution enables the straightforward interpretation of MALDI-TDS data. Whether protein identification by Mascot database searching of MALDI-TDS or T³-Sequencing spectra is needed, the direct detection of aberrations from defined reference sequences (1; shown in the left panel) or even Top-Down de novo Sequencing (2), Bruker’s ProteinScape software permits all of these approaches and keeps your data neatly organized in your project structures.

One of the most exciting recent developments in MALDI-TOF is the technology to generate images of individual molecules in tissue sections. This has the potential to revolutionize the discovery of protein targets associated with disease or treatment prognosis. MALDI imaging can also be used to monitor the distributions of lipids, drugs or their metabolites throughout the tissue without the need for labeling. With its kHz speed and 20-50 µm image resolution, the autoflex speed series was specifically optimized to deliver outstanding results in truly High Definition MALDI Imaging.

**Read more:** Rauser et al. (2010). Classification of HER2 receptor status in breast cancer tissues by MALDI imaging mass spectrometry. JPR 9(4):1854-63.

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**Protein distributions in tissue**

High resolution MALDI image of lipids and glycosphingolipids in mouse cerebellum obtained at 25 µm image resolution. Sulfatide 22:0 (m/z 862.9; green), GM2 d18:1/C18:0 (m/z 1383.4; red), and m/z 1045.8 (yellow) are covisualized in the white and gray matter.

Protein imaging shown on a horizontal rat brain section: Sinapinic acid matrix was applied using the ImagePrep\textsuperscript{TM}. Analysis was performed at 80µm lateral resolution on the autoflex speed in linear mode. Due to the unique 1,000 Hz smartbeam technology, the large (>1.5 cm\textsuperscript{2}) section could be analyzed in 6 h and with minimal harm to the tissue, allowing post-acquisition H&E staining of the section. Co-registration of a high-definition microscopic image in flexImaging\textsuperscript{TM} 3.0 allows clear alignment of molecular and anatomical features.

**smartbeam-ll technology**

The further development of smartbeam provides dramatically increased performance. Repetition rates of up to 2,000 Hz can now be achieved with a modulated beam profile (A, B). The smartbeam enables a high repetition rate of shots on identical spot positions of 30 MS/MS spectra and more. **Read more:** Holle et al. (2006). Optimizing UV laser focus profiles for improved MALDI performance J. Mass Spectrom. 2006; 41: 705-716
Glycoprotein and Glycan Analyses

Turning analysis of the complex into routine

Analysis of polymers and large proteins has always been challenging for mass spectrometry. The need for analysis of these molecules has even increased with the development of Biotherapeutics that often contain glycans or are PEGylated. To address these challenges, the autoflex speed has been developed with a number of enhancements that generate convincing results.

Glycopeptide analysis in one hit

N-linked glycopeptide from the heavy chain of a monoclonal antibody (right). The MALDI-TOF/TOF functionality offers unique glycopeptide elucidation capabilities. From a single MS/MS spectrum it is possible to identify the aglycon sequence, localize the glycosylation site and provide a glycan structure assignment – all from just a trypsin digestion.

GlycoQuest™, which is part of our ProteinScape package offers an industry first for automated glycan analysis. Glycans or glycopeptides and their isoforms can be automatically detected, localized and identified.

smartbeam-II performance

smartbeam-II laser technology provides maximum signal at minimum sample consumption. Even after a total of 364,000 shots and 100 MS/MS spectra smartbeam-II still extracts high intensity MS/MS spectra from the same spot to ensure successful peptide identification from complex samples.

High molecular weight proteins

High MW measurement of the 350 kDa protein α-macroglobulin: Superb S/N ratio due to FlashDetector™ technology. Detection of small molecules with excellent resolution to high MW macromolecules, performed in both cases by FlashDetector.

MALDI TOF/TOF spectrum of N-linked glycopeptides provides fragmentation both of the peptide part and the glycan part. A specific fragmentation pattern allows for automatic determination of the (M+H)+ of the pure peptide; the significant information for successful analysis of glycopeptides. Using this information, successful identification of the glycan and of the protein is possible with a single MS/MS-spectrum.
Bruker’s unique TLC-MALDI solution hyphenates thin-layer chromatography (TLC) and MALDI-TOF MS extending the MALDI productivity to lipid profiling, herbals, cosmetics and food analysis.

TLC plates can be coated in MALDI matrix within a few minutes permitting the molecular high resolution readout of entire TLC lanes. Molecular TLC-images or chromatograms are generated by dedicated software tools and bring the identification of compounds on the TLC-plate to your fingertips.

The MALDI Biotyper for identification and classification of microorganisms is a revolutionary approach to definitive identification and speciation of microorganisms.

Harnessing the power of mass spectrometry for an innovative application, the MALDI Biotyper system provides a fast, accurate, high value system that is easy to use to provide crucial information for clinical laboratories. Virtually reagent-free and proven to be superior to traditional biochemical methods in many ways, the MALDI Biotyper is blazing a trail in healthcare around the world.

Triplicate TLC-MALDI-TOF analysis of lipid mixture

HPTLC-MALDI analysis of a 5 lipid mixture. 3 repetitive analyses with 330 ng lipid standard on 2 TLC plates were analyzed by densitometry (left) and by MALDI (center). The extracted ion chromatograms of all 5 lipid species show a good robustness of the TLC-MALDI method (right). The MALDI imaging readout of the same data provides clear molecular assignments even in the case of overlapping bands.
Applications in the field of synthetic polymers require equally good performance in both, small to medium mass range as well as high mass analysis. The autoflex speed series is well prepared for either of these tasks: Being equipped with the latest FlashDetector technology, the instrument provides outstanding resolution (R>26,000) and mass accuracy (≤ 2 ppm) in reflectron mode to address the complexity of e.g. complex co-polymer samples. Due to its real on-axis detector design, operating the system in linear mode allows the detection of extremely large molecules such as polymers >500 kDa (top).

Characterization of a polyethylene oxide-polypropylene oxide (PEO-PPO) co-polymer by liquid adsorption chromatography (LAC)-MALDI: LC separation was performed under near-critical conditions for propylene oxide units. The eluate was mixed with DCTB matrix on the fly and sprayed onto the MALDI target.


FlashDetector

autoflex speed delivers mass accuracy ≤ 2 ppm and maximum resolving power >26,000 in reflectron mode due to its patented PAN™ technology and unique FlashDetector. In linear mode, ions with molecular weight >500 kDa can be detected using the unique FlashDetector.
Technical Specifications

Increased efficiency in MALDI-TOF (/TOF)
- 2 kHz smartbeam-II laser technology enables ultra-high data acquisition speed.
- Laser focus diameters down to 10 µm for high spatial resolution imaging without pixel overlap.
- Wide mass range resolving power up to 26,000 due to proprietary PAN™ technology.
- FlashDetector™ combined with new 4 GHz digitizer provides 2 ppm mass accuracy for highest confidence.
- MALDI Perpetual™ Ion Source with laser-based self-cleaning in minutes. Its push-button concept for removing sample and matrix debris is indispensable for KHz operation.

Compass software suite
- flexControl for quick and easy data acquisition.
- flexAnalysis for automatic and interactive data analysis.

Tailored application-specific software packages
- ProteinScape™ for comprehensive handling of proteomics projects.
- GlycoQuest: Automated detection, localization and identification of glycan isoforms
- BioTools™, the cutting edge software for protein analysis and Top-Down sequencing
- flexImaging™, the leading platform for MALDI Imaging.
- ClinProTools™, statistical model generation software; turns images into interpreted data.
- PolyTools™, interactive interpretation of MALDI polymer spectra.

Additional supported software tools
- Polymerix (Sierra Analytics, Modesto, CA, USA)
- SimGlycan (PREMIER Biosoft International, Palo Alto, CA, USA)

Support features
- Extended self diagnostics.
- Remote on-line service and support capability.
- IQ/OQ/PV procedures for regulated environments.
- Maintenance contracts – various options available.

For research use only. Not for use in diagnostic procedures.

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