



## Technical Note # TN-31

# Redefining MALDI-TOF/TOF Performance

The new ultrafleXtreme™ exceeds all current expectations of MALDI-TOF/TOF technology: A proprietary kHz smartbeam-II™ MALDI laser integrated with a novel FlashDetector™ and re-engineered electronics makes it the only MALDI TOF/TOF on the market to provide kHz acquisition in MS and MS/MS. It generates a new level of data quality in applications such as LC-MALDI proteomics, high resolution tissue imaging based biomarker discovery or Top-Down Sequencing.

### Introduction

This next generation, genuine 1 kHz MALDI instrument sets new standards with an unprecedented performance level. It brings kHz laser repetition rates to all high-throughput applications, from quantitative proteomics workflows utilizing labels (such as ICPL 4plex, SILAC or iTRAQ 8plex) to label-free biomarker discovery directly from tissue samples. At the same time, resolution and mass accuracy define a new standard for MALDI-TOF.

#### Resolving Power:

Advanced electronic engineering and a unique Flash Detector combined with 4 GHz digitization rate at full spectra length and proprietary “panoramic” PAN technology provides high resolving power >40,000 in the peptide mass range (Fig. 1, Fig. 2) and beyond (Fig. 3). This new

level of performance leads directly to increased numbers of identified proteins in LC-MALDI at improved specificity.

#### Mass Accuracy:

Superb resolving power combined with, sophisticated calibration routines yields unrivaled ppm mass accuracy on the ultrafleXtreme for both internally and externally calibrated spectra. Power supplies with excellent temperature stability and enhanced high voltage pulse electronics for kHz repetition rates result in new levels of data quality (Fig. 4).

#### Speed & Sensitivity:

The proprietary smartbeam™-II laser with 1000 Hz repetition rate was specifically developed in-house for the ultrafleXtreme MALDI-TOF/TOF. The acquisition control software allows operation from 1 to 1000 Hz repetition frequency in MS as well as in MS/MS mode. Therefore, kHz data acquisition of high resolution tissue protein images and LC-MALDI analysis in Biomarker workflows are both perfectly sustained on the ultrafleXtreme (Fig 5).

In addition, the optimized smartbeam-II laser delivers constant high output energy, regardless of the selected repetition frequency, across long acquisition runs. This is indispensable for a series of MALDI imaging runs or LC-MALDI runs.

## High mass resolution available

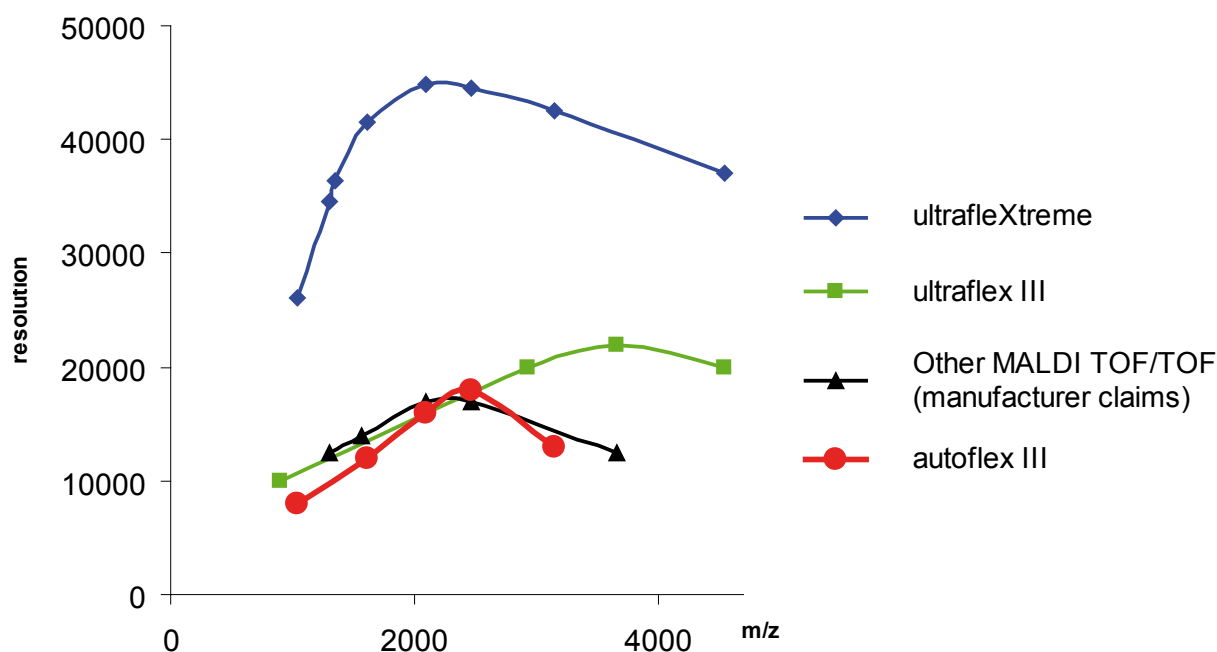


Fig. 1: The ultrafleXtreme provides superb resolution and specificity. This plot of resolving power as a function of  $m/z$  shows its impressive performance across the relevant peptide mass range.

In addition to speed, Bruker's proprietary next generation smartbeam-II™ laser provides increased performance due to highly refined focal beam patterns [1-3]. This is essential for optimum sensitivity across a wide range of applications. It can be applied to all commonly used MALDI matrices and with all sample preparation techniques such as thin-layer and dried droplet. The high pulse energy of  $>85 \mu\text{J}$  at 1 kHz ensures high confidence results in peptide identification and quantitation as well as unparalleled performance in MALDI top-down sequencing (MALDI-TDS) of intact proteins.

### The ultimate tissue imaging technology

A computer-controlled variable laser focus diameter of 10 - 100  $\mu\text{m}$  allows new levels of spatial resolution – essential for emerging applications like high resolution MALDI tissue imaging. Achieve incredible spatial resolution with no loss of protein signal intensity (Fig. 6). The life of our smartbeam-II laser extends to  $>10^9$  laser shots and outperforms any other laser, reducing down time and maintenance costs.

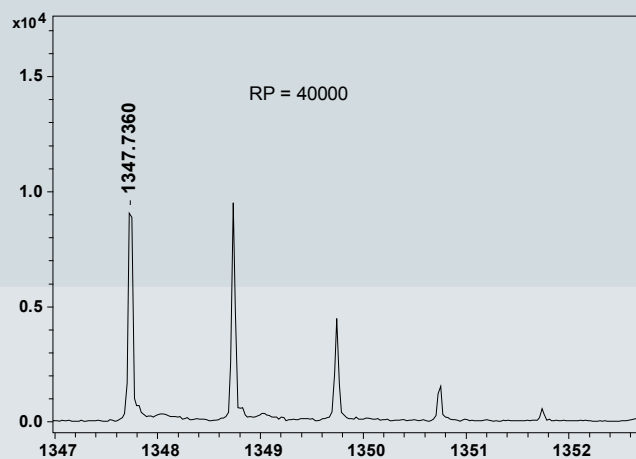
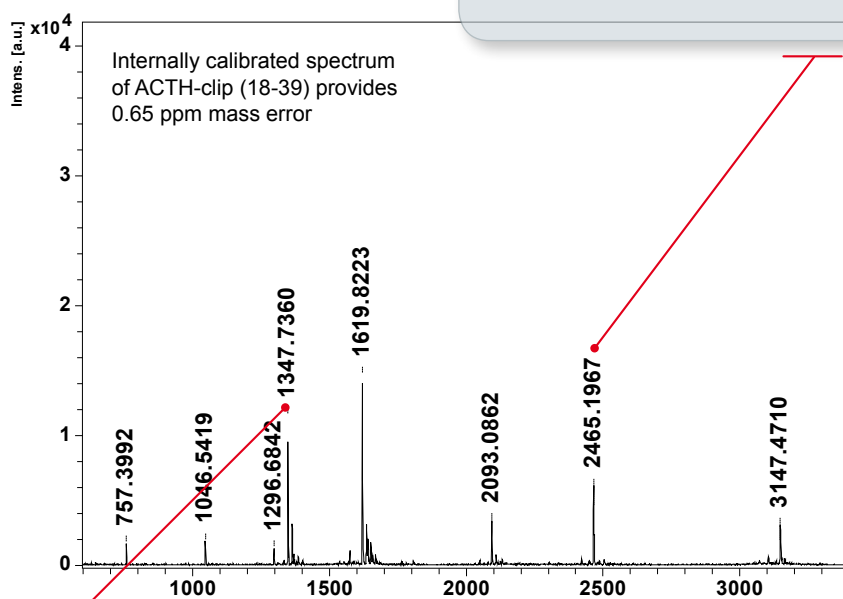
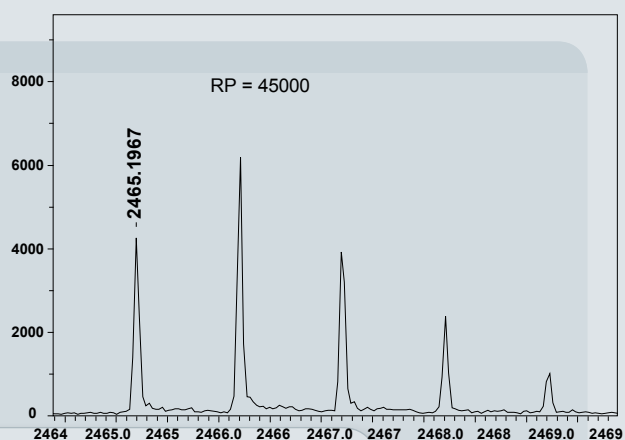
The new wide-range PAN resolution provides  $>40\text{k}$  resolving power, which is three times higher than previous instruments. This high resolving power improves accuracy by separating overlapping peaks and enables to unambiguously identify components in the typically crowded region of MALDI TOF spectra @900Da (Fig. 7).

### MALDI Perpetual™ ion source with laser-cleaning in minutes

The MALDI Perpetual ion source implements an entirely new design for the removal of sample and matrix debris. Its push-button, laser-cleaning concept is indispensable for kHz operation – and it works for all MALDI matrices. The self-cleaning ion optics are ready for the next run within a few minutes (Fig. 8).

## Increased resolution and mass accuracy

Fig. 2: ultrafleXtreme delivers mass accuracy < 1 ppm and superb resolving power >40,000 simultaneously across the Proteomics mass range due to its patented PAN™ technology.



## Market-leading technology

The combination of technologies implemented results in unmatched performance and productivity from the ultrafleXtreme MALDI-TOF/TOF:

- Bruker's patented 1 kHz smartbeam-II laser provides focus diameters down to 10  $\mu\text{m}$  for high resolution imaging near the single cell level, at constant signal intensity even at small focus diameters.
- 1 kHz electronics engineered with precision high voltage control brings the laser performance to a true systems performance for all operational modes. From protein tissue imaging to LC-MALDI proteomics work, all fully enabled at 1-1000 Hz data acquisition rate for both TOF and TOF/TOF operation.
- Proprietary PAN technology for high mass accuracy and resolution across a wide mass range, for precision proteomics and Top-Down protein sequencing in recombinant protein work (Fig. 9).

- The new FlashDetector provides unmatched mass resolution and accuracy for data you can trust.
- The new 4 GHz digitizer, coupled with dedicated, re-engineered electronics, transforms the ultrafleXtreme into a high performance mass analyzer with unmatched system performance.
- The proprietary self-cleaning MALDI Perpetual ion source complements the suite of innovations ensuring robust operation. Long laser life in combination with automated laser-based source cleaning in minutes underpins a high-duty cycle and long service intervals.

## Conclusion

Along with being easy to use and simple to maintain, the ultrafleXtreme delivers unmatched speed, high performance in MS and MS/MS modes, and efficiently provides the full power and flexibility of MALDI-TOF-TOF for a wide range of applications.

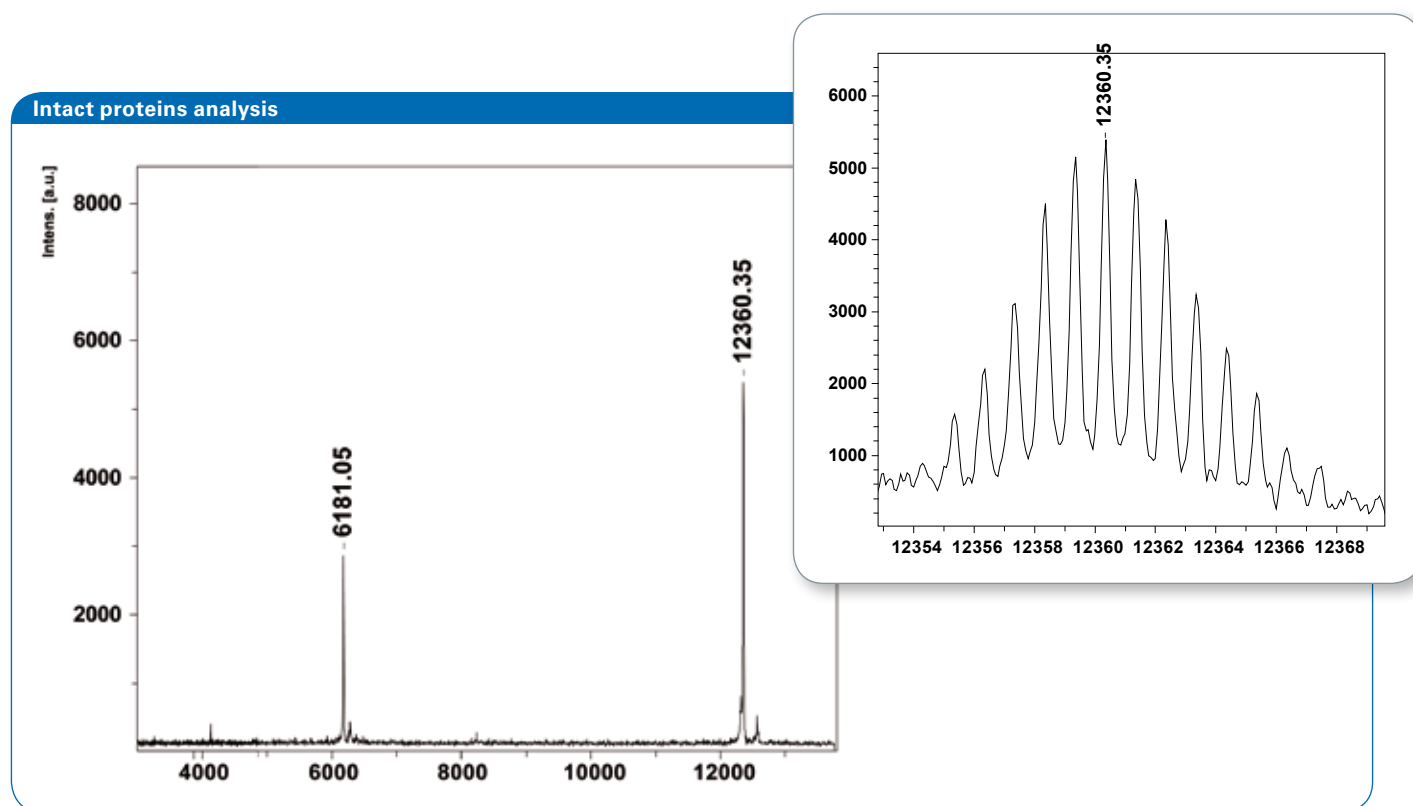
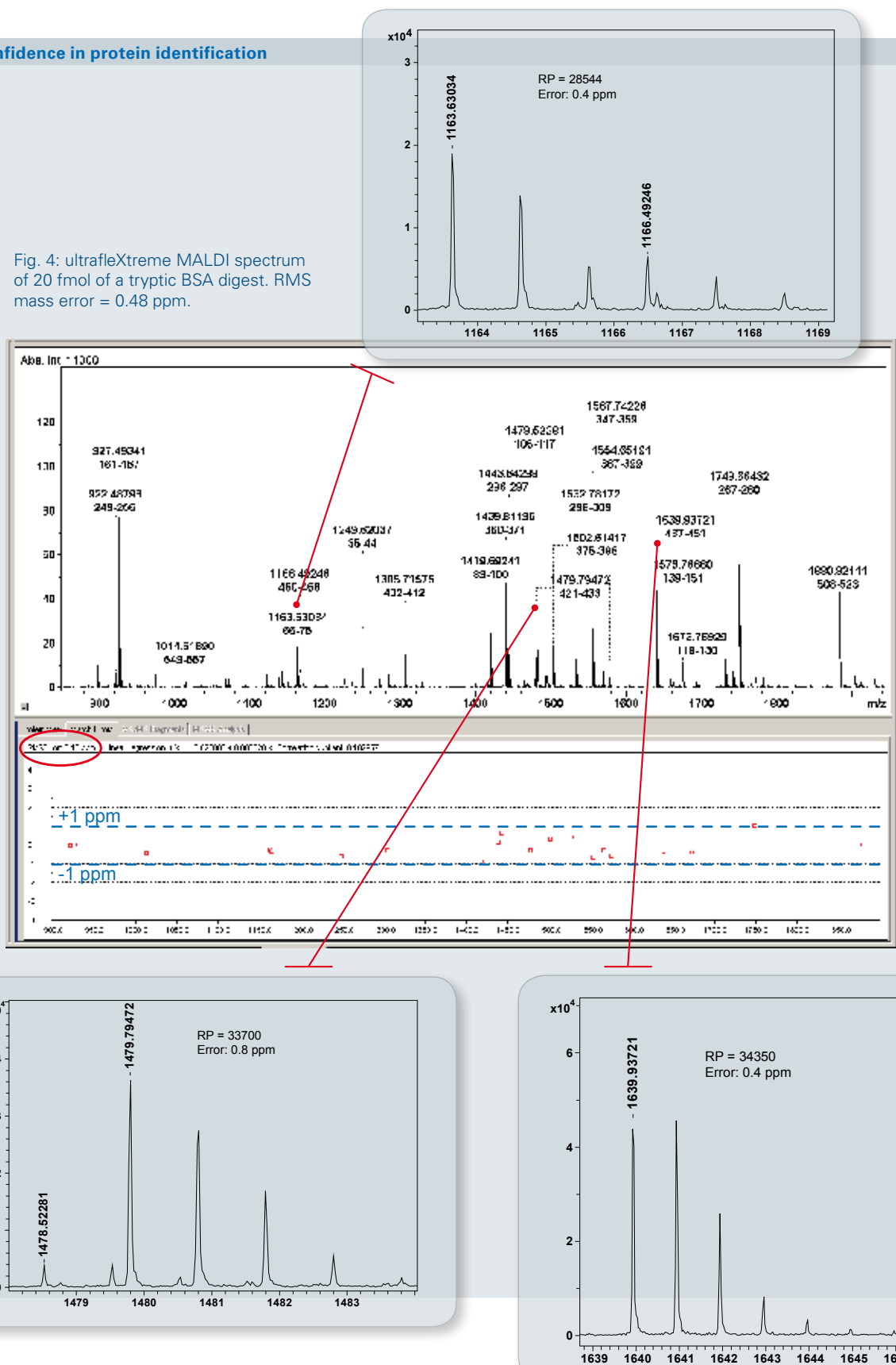


Fig. 3: ultrafleXtreme provides isotopic resolution even for intact proteins at 12 kDa (here: cytochrome c).

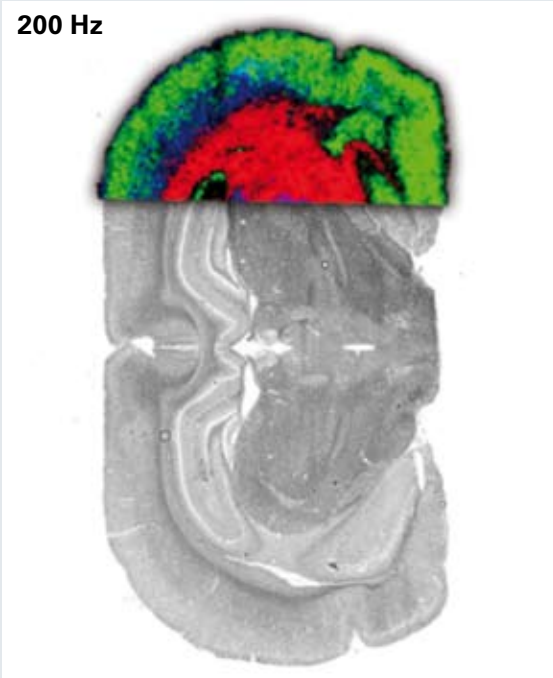
## High confidence in protein identification

Fig. 4: ultrafleXtreme MALDI spectrum of 20 fmol of a tryptic BSA digest. RMS mass error = 0.48 ppm.



## The speed advantage of 1 kHz imaging

200 Hz



1000 Hz

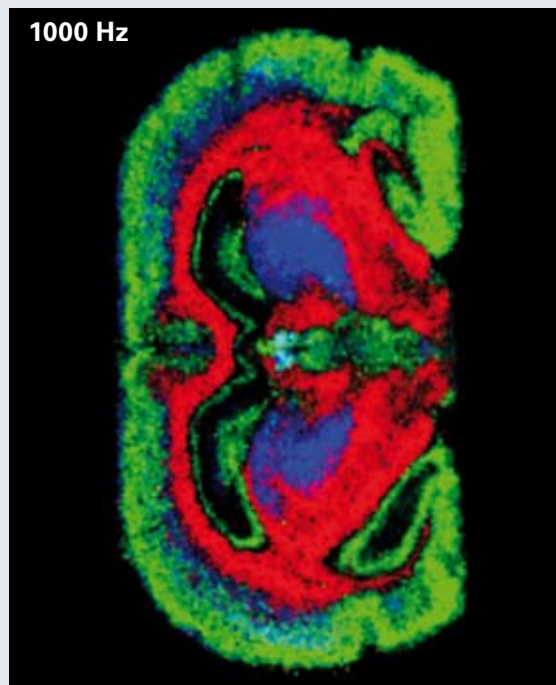


Fig 5: kHz acquisition on ultrafleXtreme (right) with 2 pixel/sec vs. classical 200 Hz acquisition (left). Within the same data acquisition time, a full image of the mouse brain can now be obtained. Sample: mouse brain sections with 10  $\mu\text{m}$  thickness. MALDI image overlaid on scanned tissue image after identical acquisition time.

## High resolution imaging of proteins is enabled by smartbeam-II technology

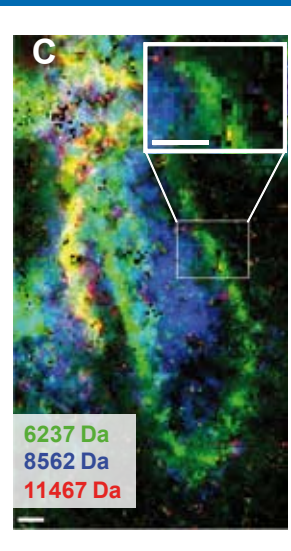
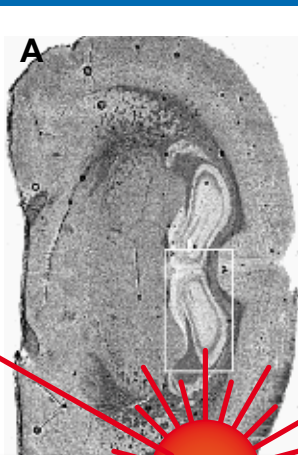
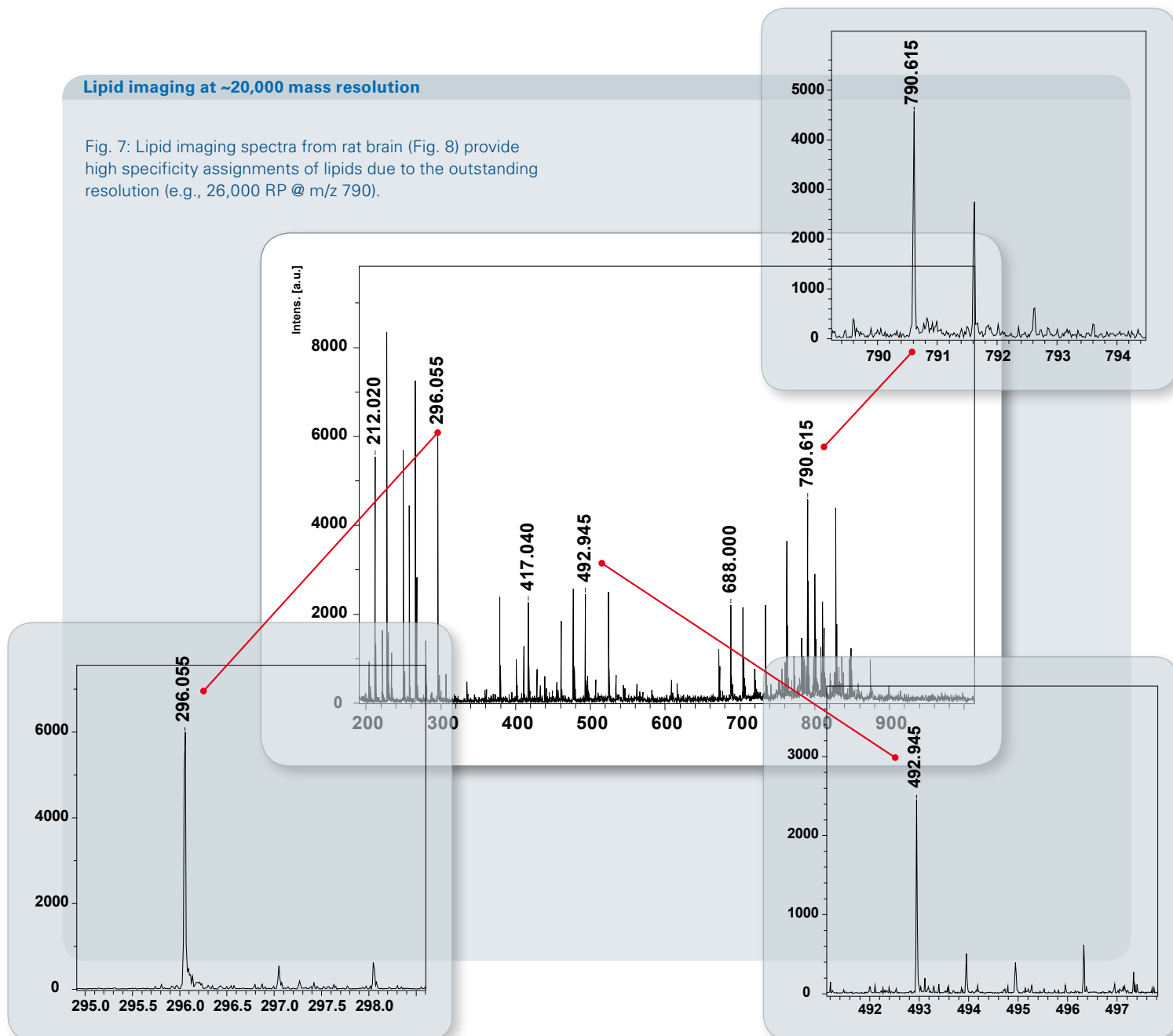


Fig 6: High resolution imaging of proteins in rat brain hippocampal formation acquired at 2 pixel/sec; scalebars 200  $\mu\text{m}$ . A) Optical image of whole rat brain; B) zoom view of area to be analyzed by MALDI; C) MALDI image with three protein mass distributions colored in red, green and blue at 20  $\mu\text{m}$  image resolution.

Mass spectra acquired directly from tissue with different smartbeam-II laser focus diameters (100, 25, 10  $\mu\text{m}$ ; from top to bottom). 200 laser shots were acquired per spectrum. Peak intensities and spectra qualities are identical independent of the laser focus setting.

## Lipid imaging at ~20,000 mass resolution

Fig. 7: Lipid imaging spectra from rat brain (Fig. 8) provide high specificity assignments of lipids due to the outstanding resolution (e.g., 26,000 RP @  $m/z$  790).



## Self-cleaning ion source based on laser-irradiation

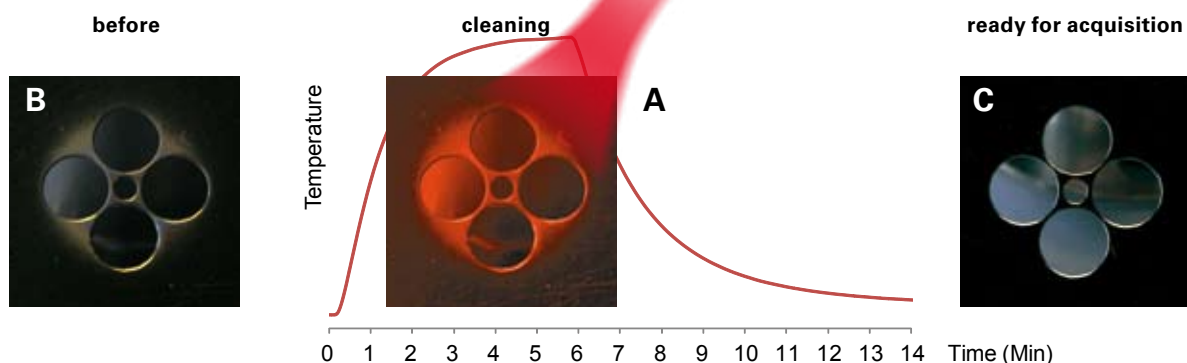


Fig. 8: The proprietary MALDI Perpetual™ ion source is automatically laser-cleaned in minutes. The unique, laser-based cleaning process removes all MALDI matrices without residues. Extraction plate temperature profile during the cleaning process (A). B. Extraction plate before and (C) after the laser cleaning process.

## Top-down protein analysis for biopharmaceuticals

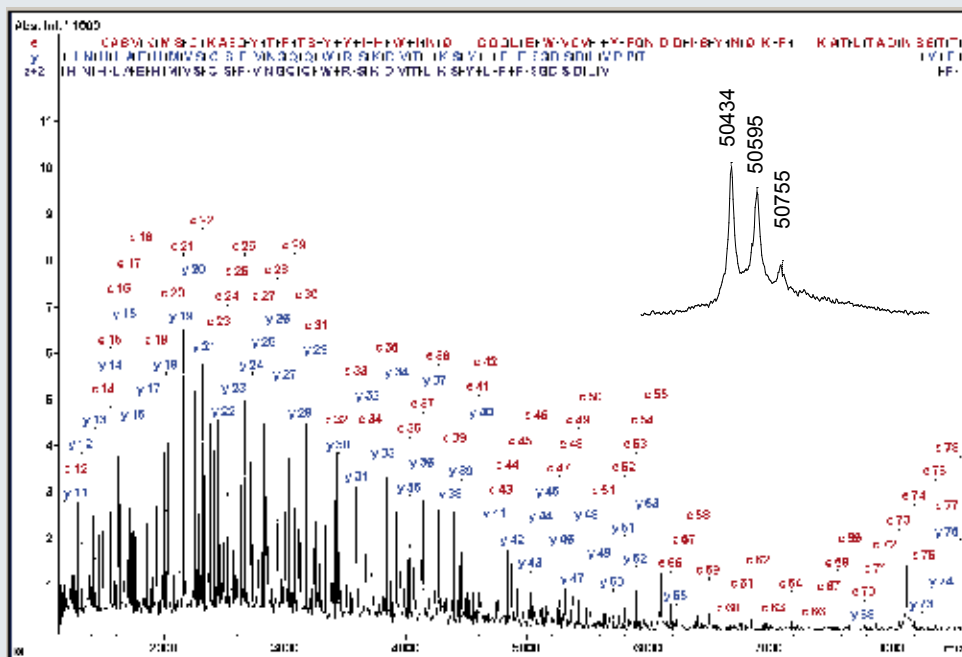


Fig. 9: MALDI Top-Down Sequencing (MALDI-TDS) spectrum of a recombinant antibody heavy chain acquired in 2 sec in an automated 1 kHz acquisition. 55 C-terminal and 77 N-terminal amino acids read out in this single spectrum and, simultaneously, the N-terminus is assigned being pyroglutamylated by a specific mass offset of the c-ion series. The MW region from an intact molecule is shown in insert, indicating glycan heterogeneity of the HC.

For further information:  
[www.bdal.com/ultrafleXtreme](http://www.bdal.com/ultrafleXtreme)

### Keywords

kHz-Laser  
resolution  
speed  
accuracy  
robustness

### Instrumentation & Software

ultrafleXtreme  
smartbeam-II  
FlashDetector

### References

- [1] Bruker Technical Note TN-15
- [2] A. Holle, A. Haase, M. Kayser & J. Höndorf, Optimizing UV laser focus profiles for improved MALDI performance (2006) J. Mass Spectrom. 41: 705-716.
- [3] Bruker Technical Note TN-31
- [4] Bruker Technical Note TN-32

### Authors

Detlev Suckau, Jens Höndorf, Anja Resemann,  
Sören-Oliver Deininger; Bruker Daltonik, Bremen, Germany.

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[www.bdal.com](http://www.bdal.com) • Bruker Daltonik GmbH

Bremen · Germany  
Phone +49 (421) 2205-0  
Fax +49 (421) 2205-103  
[sales@bdal.de](mailto:sales@bdal.de)

Bruker Daltonics Inc.

Billerica, MA · USA  
Phone +1 (978) 663-3660  
Fax +1 (978) 667-5993  
[ms-sales@bdal.com](mailto:ms-sales@bdal.com)