SHIMADZU
Excellence in Science

Technical Report

No.MO373

MALDI-7090

MALDI Mass Spectrometer

Achieving Ultimate MS/MS Resolution in a MALDI TOF-TOF using ASDF – Axial Spatial Distribution Focussing

The MALDI-7090™ is a MALDI TOF-TOF mass spectrometer capable of delivering high-energy CID (20 keV lab collision energy) MS/MS analysis. This fragmentation is induced through high-energy collisions with neutral gas molecules. However, for efficient CID with a MALDI ion source, the laser power still has to be above threshold level. An immediate effect of increasing the laser power for MS/MS acquisitions is that the resolution of both the precursor and product ions is decreased. This reduction in mass resolution has its origins in the increase in the various distributions produced, namely the velocity and spatial distribution of ions in the axial and radial directions. As shown in figure 1, the majority of these can be compensated for using either pulsed extraction or the ion optics. Nevertheless, it is well known that only one axial distribution can be focussed at a time i.e. it is not possible to compensate simultaneously for both spatial and velocity distributions using pulsed extraction alone[1].

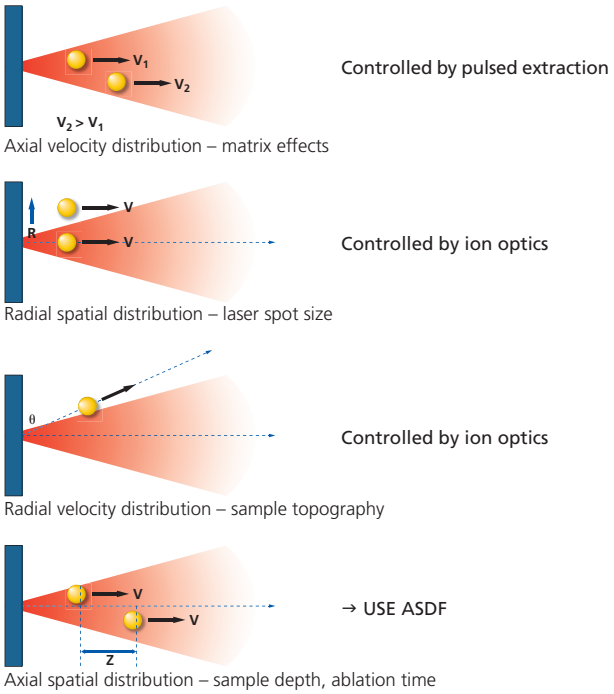

Figure 1: Distributions affecting mass resolution and how they are controlled

The MALDI-7090 incorporates a novel patented technique to compensate for the axial spatial distribution of the ions formed during MS/MS without affecting the other distributions such as velocity distribution. This method is termed ASDF™ (or Axial Spatial Distribution Focussing) and in the MALDI-7090 provides ultimate resolution in MS/MS mode (10 000 FWHM).

The graphs in figure 2 illustrate the effect of applying ASDF to MS/MS acquisitions. The first graph (2a) shows that for an acquisition with pulsed extraction but without ASDF applied, the peak width contribution at the reflectron focus in the flight tube due to axial velocity distribution (red trace) is almost nil. However, the peak width contribution at the same point due to axial spatial distribution (blue trace) is of the order of several tens of nano seconds thus reducing the peak resolution. On the other hand, the second graph (2b) shows that for a similar acquisition with both pulsed extraction and ASDF applied, the peak width contributions from both the axial spatial and axial velocity distribution are virtually nil allowing very high MS/MS resolution to be achieved.

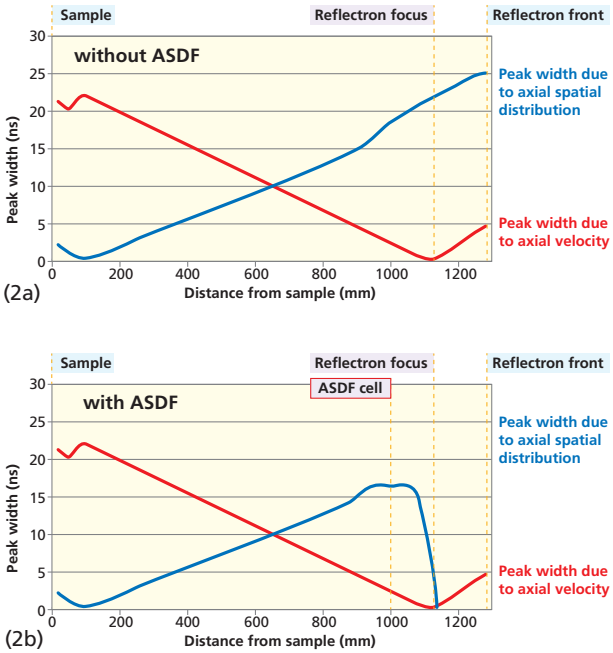

Figure 2: Effect of applying ASDF on peak width

ASDF works by applying a pulsed axial electrostatic field to the precursor and fragment ions in the field-free region immediately before they enter the reflectron. This electrostatic field has the effect of focussing the axial spatial distribution of the ions without affecting the velocity distribution (which has already been focussed using pulsed extraction from the ion source).

The result of applying ASDF in addition to pulsed extraction during an MS/MS acquisition is illustrated in figure 3. The full MS/MS spectrum shown in the inset demonstrates almost complete sequence coverage of the detected fragment ions. The detailed region (m/z 1190 - 1310) shows the high resolution achieved using ASDF (10 000 FWHM) as well as the presence of high-energy w-type fragment ions characteristic of side chain fragmentation.

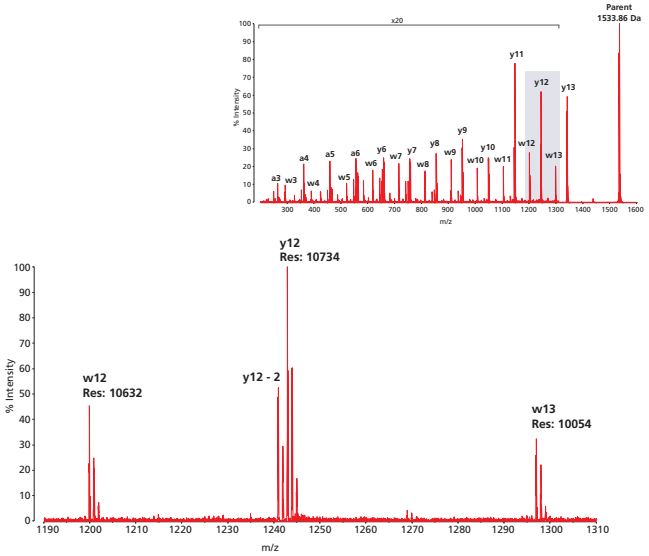

Figure 3: Detail of the MS/MS spectrum for P14R peptide (m/z 1533.8) with ASDF applied. Inset: full mass range.

More interestingly a y-2 ion (or Y-ion) is also observed. This ion species is also characteristic of high-energy fragmentation and occurs by H-transfer away from the C-terminal fragment to form the structure $\{NH{=}CR_{n-i}\ldots\text{-}NH\text{-}CHR_n\text{-}COOH\}H^+$. Under high-energy collision conditions, proline residues are known to produce abundant (y-2)-type ions, i.e. they feature a doublet separated by 2 Da such as that observed in figure 3. In lower resolution MS/MS acquisitions without the benefit of ASDF, this doublet may not be clearly observed as the isotopic distributions overlap and may therefore cause a shift in the fragment mass reported.

A further advantage of using ASDF is that the resolution of the MS/MS spectrum becomes largely independent of the laser fluence used. The spectra in figure 4 illustrate this effect, showing three increasing laser powers used in the MS/MS analysis of P14R peptide: threshold (a), 120% threshold (b) and 140% threshold (c). The resolution obtained for the fragment ions remains essentially unaffected by the laser fluence.

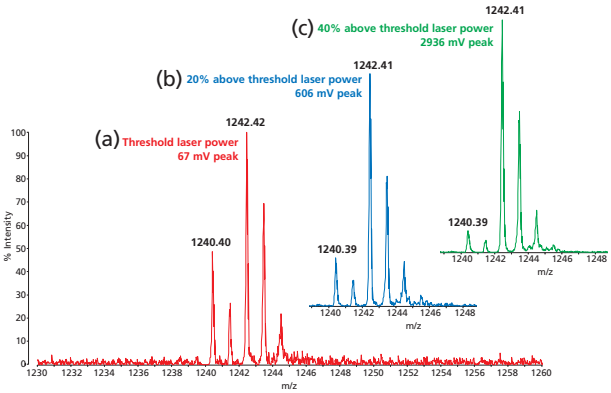

Figure 4: Laser fluence independence of the MS/MS mass resolution using ASDF

Conclusion

- ASDF is a unique patented method for ultimate MS/MS ion resolution in MALDI TOF-TOF
- ASDF focusses the axial spatial distribution with very high efficiency
- Mass resolution 10 000 FWHM can be achieved for MS/MS acquisitions using ASDF
- With ASDF, MS/MS resolution is largely independent of laser fluence used

References

(1) Wiley WC, McLaren IH. *Rev. of Sci. Instrum.* 1955; **26** (12): 1150.

First Edition: May 2013

Kratos Analytical Ltd.
Wharfside, Trafford Wharf Road, Manchester M17 1GP, UK
Phone: +44 161 888 4400 Fax. +44 161 888 4402
URL http://www.shimadzu.com/an/