

## Low Mass Zoom™ (LMZ)

MALDI TOF-TOF instruments make use of a collision gas to aid fragmentation. High energy MS/MS spectra generated from AXIMA-TOF<sup>2</sup>™ provide additional structural information through side-chain fragmentation (d/w-ions). Also, diagnostic low mass fragment ions including immonium ions and several other important indicative ions to aid in sequence elucidation may be found in the spectra. For certain applications it can be advantageous to achieve the highest possible resolution and sensitivity in this low mass region. The AXIMA-TOF<sup>2</sup>™ mass spectrometer supports a novel MALDI TOF-TOF analysis mode, Low Mass Zoom™ (LMZ), which enhances this valuable information and provides a powerful investigative tool. In this mode, low mass ions are better focused by automatically tuning voltages on the curved field reflectron, resulting in increased sensitivity and higher resolution in the low mass region.

This approach provides multiple benefits adding further confidence to ion series interpretation by emphasizing tryptic peptide low mass ions and aiding de-novo sequencing or extrapolation of the ion series. Additionally, this feature can also be used to focus analysis for targeted applications such as iTRAQ™ multiplexed relative quantification chemistry.

Using LMZ mode, 5-fold increases in sensitivity can be achieved for low mass fragment ions generated from parents with masses above 1800 Da. Additionally, fragment ion resolution can be improved by up to 4-fold, depending on the precursor ion mass.

The LMZ button is located on the Firing panel of the LAUNCHPAD™ software acquisition window (Figure 1) and is unavailable for use during MS mode. LMZ mode is an extension of MS/MS mode and so may only be selected when the instrument is configured for an MS/MS acquisition (with the ion gate and the CID gas both on).

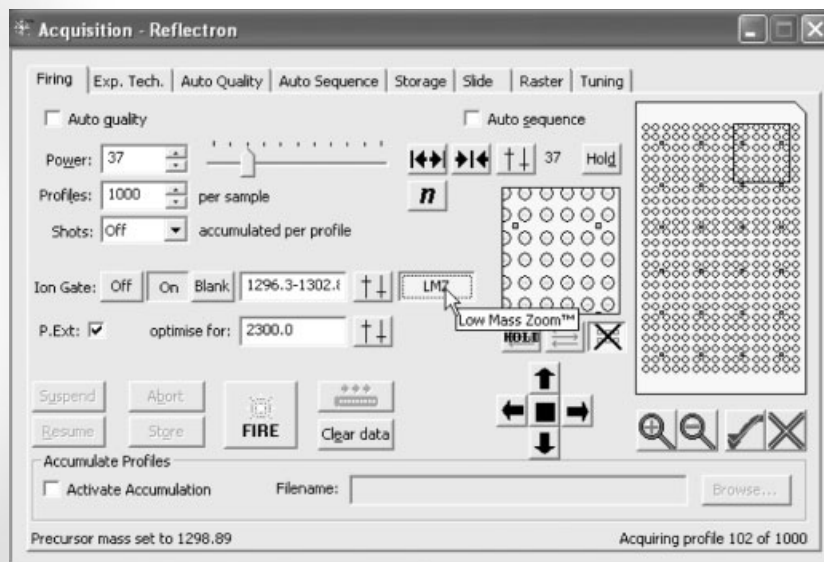


Figure 1. Firing tab displaying LMZ functionality

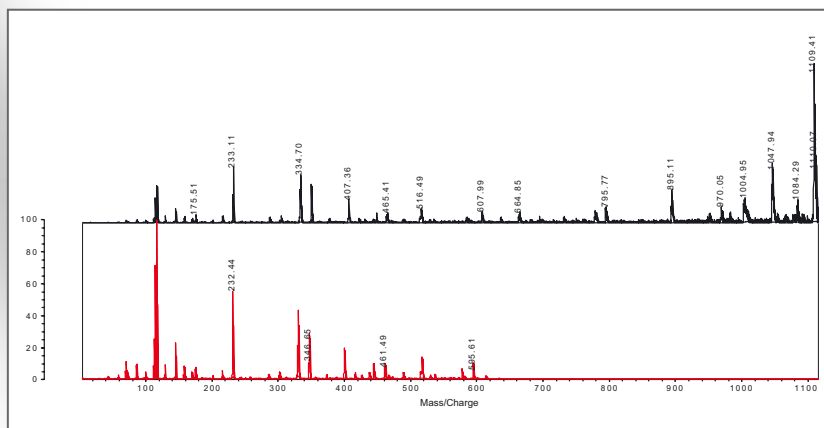


Figure 2. MS/MS and LMZ spectra of iTRAQ™ labelled precursor ion of  $m/z$  1126

Figure 2 shows a comparison of the spectra obtained when fragmenting an iTRAQ™ labelled peptide ion using MS/MS mode and LMZ mode. The low mass region is particularly important for such analyses where ions within the 114-117 Da region are used for quantification purposes. An increase in intensity at the low mass fragment ion region is clearly shown; the signal-to-noise ratio is also significantly improved.

In an expanded view of the 80-160 m/z region (Figure 3), the increases in ion intensity and resolution when using LMZ mode are highlighted further. The LMZ functionality is also appropriate for aiding sequence determination by highlighting the immonium ions present. An example is shown in Figure 4.

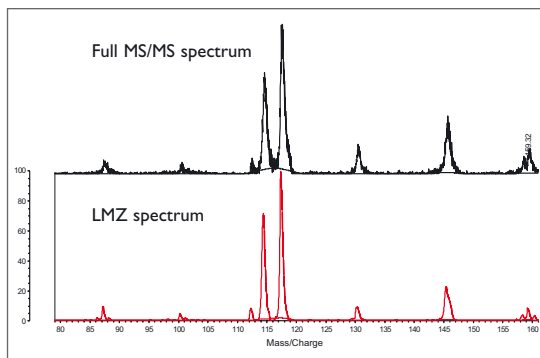


Figure 3. Expanded view (80-160 m/z) of MS/MS spectra of iTRAQ labelled precursor ions (1126 m/z)

## Conclusions

- LMZ functionality can significantly improve low mass fragment ion sensitivity and resolution
- Used to aid sequence determination utilizing immonium ions
- Can dramatically enhance the quality of reporter ion data required for iTRAQ™ applications

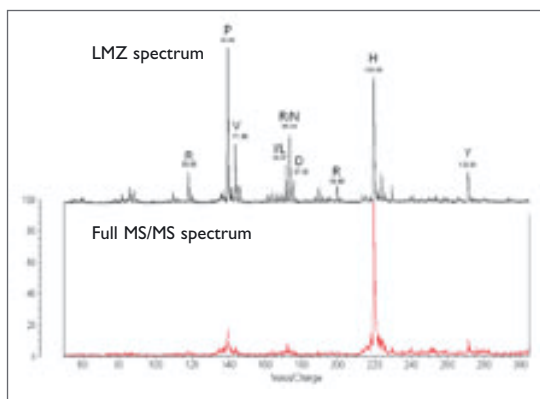


Figure 4. MS/MS and LMZ spectra of Angiotensin II expanded view of immonium ion region