Improving MALDI-TOF Performance with Practical Implementation of Very High Post-Acceleration

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Overview:

Purpose:

To improve MALDI-TOF performance for ion masses > 100 kDa using the practical implementation of very high post acceleration voltages.

Methods:

Measurement of MALDI-TOF spectra of standard high-mass samples using a new modular detector capable of operation at very high voltages (30 kV) using a state-of-the-art MALDI-TOF instrument.

Results:

Good MALDI-TOF spectra can be collected with a simple MCP-based detector for ions with masses > 100 kDa even with only modest ion energy (20 keV). The use of very high post acceleration voltages is not necessary provided that the creation and transport of ions is good and the detector is capable of handling the ion flux.

Introduction:

MALDI-TOF:

There is theoretically no upper mass limit for TOF. At higher masses, the instrument is typically limited by the ion detector.

Detection of High Mass Ions:

For electron-multiplier ion detectors such as microchannel plates (MCPs) or discrete dynode detectors, the detection of an incoming ion depends on the emission of at least one secondary electron at the detector input.

The probability of kinetic secondary electron emission is approximately proportional to the ion's velocity, *v*. The detection efficiency of MCPs for high mass ions has been approximated in the literature as being proportional to $(E/M)^{1.75}$ (Twerenbold et al., 2001)

In TOF systems ions are accelerated to essentially a single kinetic energy, *E*.

The velocity of the ions in the TOF flight tube is therefore proportional to the square root of the mass per charge $m/q^{1/2}$, making electron-multipliers less sensitive to high mass ions.

$$v \cong \sqrt{\frac{2E}{\left(\frac{m}{q}\right)}} \qquad \begin{matrix}\textit{Detection}\\\textit{Efficiency}\end{matrix} \sim v \qquad \begin{matrix}\textit{Detection}\\\textit{Efficiency}\end{matrix} \propto \left[1 - e^{-1620\left(\frac{E\,(keV)}{M\,(Da)}\right)^{1.75}}\right]$$

Typical Methods for Improving High Mass Operation:

Post acceleration of the ions after flight, increases the ion impact energy.

Surface coatings on the input surface of the detector increase the probability of electron emission.

Fragmentation of target molecules followed by accelerations of the product ions.

Use of alternate detector technology (e.g. cryogenic superconducting tunnel junctions [STJ]), which do not have a velocity-dependent detection efficiency.

Novel Aspect of this Study:

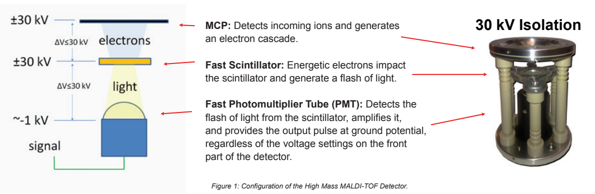
Optically-coupled detector capable of very high isolation voltage (30 kV).

Detector allows MALDI-TOF instrument to be run in many operational modes without physical changes to the detector.

Looking for a simpler and lower-cost approach to high-mass detection that does not result in a loss of resolving power.

Methods:

Detector Configuration:



MCP: Detects incoming ions and generates an electron cascade.

Fast Scintillator: Energetic electrons impact the scintillator and generate a flash of light.

Fast Photomultiplier Tube (PMT): Detects the flash of light from the scintillator, amplifies it, and provides the output pulse at ground potential, regardless of the voltage settings on the front part of the detector.

Figure 1: Configuration of the High Mass MALDI-TOF Detector.

Detector Requirements:

High Gain:
Three gain stages can be balanced depending on the application.

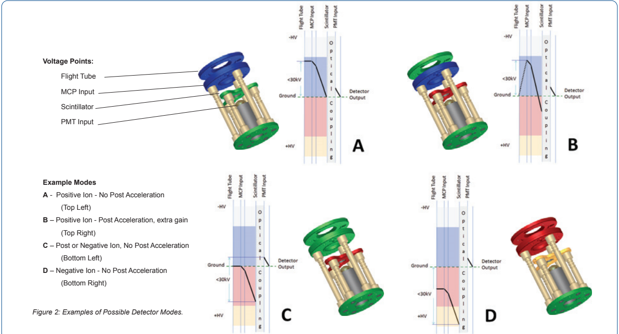
The light output of the scintillator is proportional to the energy lost to electronic collisions – so high isolation voltages can translate into high light output.

High Dynamic Range:
Large populations of low mass ions can saturate the detector, blinding it to the higher mass ions. Low voltage operation of the MCP and a high output current PMT allows high dynamic range and linear output pulses > 2 V into 50 Ω.

Low Noise:
High light output for single-ion pulses far exceeds digitizer electronic noise or any noise due to thermionic emission of electrons from the PMT photocathode.

Sensitivity:
Thin insulating coatings on the input surface dramatically improve kinetic electron emission for slow ions. (e.g. Ricardi, et al., 2004)

Methods (continued):



Example Modes
A - Positive Ion - No Post Acceleration (Top Left)
B – Positive Ion - Post Acceleration, extra gain (Top Right)
C – Post or Negative Ion, No Post Acceleration (Bottom Left)
D – Negative Ion - No Post Acceleration (Bottom Right)

Figure 2: Examples of Possible Detector Modes.

MALDI-TOF Mass Spectrometer:

Spectra were collected on a SimulTOF 100 Linear MALDI-TOF instrument (Figure 3, right) which uses a new simultaneous space and velocity focus method and gridless ion optics.

Figure 3: SimulTOF Linear MALDI-TOF.

Results:

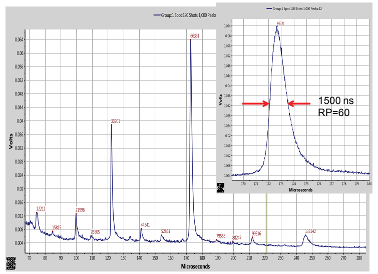


Figure 4: 100-Shot average spectrum of BSA in Sinapinic acid matrix. The peak width for singly-charged ion is 1500 ns corresponding to a resolving power of 60. The ion energy is 20 kV and the MCP input surface is maintained at ground potential. (Mode C in Figure 2, top.) The data does not include any form of background subtraction. The fact that clean high-mass spectra can be obtained with no post-acceleration at modest ion energy negates the need for the very high post acceleration, which can produce artifacts in the spectrum. The ability to put very high voltage on the scintillator and operate the MCP at low gain results in good sensitivity and high dynamic range.

Results (continued):

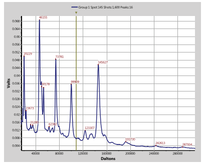


Figure 5: 1600-Shot average spectra of IgG in Snapinic acid matrix show good sensitivity to masses > 100 kDa. The ion energy is 20 kV and as in Figure 4, the MCP input surface is operated at ground potential with no post-acceleration.

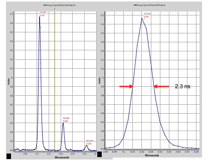


Figure 6: Low mass results. Spectrum of α-cyano matrix dimer showing in-system peak width of 2.3 ns in SimulTOF 100 Linear MALDI-TOF. The ion energy is 15 keV. The resolving power is ~3300.

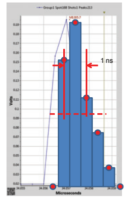


Figure 7: Low Mass Results. Histogram of digitizer output for a low-mass single ion peak using the detector equipped with a recently developed fast scintillator, showing a peak width (FWHM) of ~ 1 ns, which is the peak width of the PMT.

Conclusions:

- High-quality spectra of ions with masses > 100 kDa can be obtained at moderate ion energies (20 keV) with a relatively straightforward and flexible combination of MALDI-TOF instrument and detector.
- This result is opposite of our initial expectation, which was that very high post-acceleration voltages would be needed to obtain high quality data.
- For the samples tested, very high post-acceleration did not produce sufficient improvements in sensitivity to outweigh its undesired effects on the mass spectra for these samples, but impact energies > 50 keV could be achieved if necessary.

References:

1. D. Twerenbold, et al., *Proteomics 1* (2001) 66.
2. P. Riccardi et al., *Surface Sci. Letters*, 571 (2004) L305

Future Work:

Further optimization of input surface coatings.

Further optimization of scintillator properties.

Possible physical reconfiguration of components based on application (Figure 8).

Figure 8: Future Work:

The detector components do not need to be directly connected.

The PMT could be outside of the vacuum system.