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Introduction
A new MALDI-TOF-TOF instrument has been developed to overcome the limitations of current tandem MALDI-TOF instruments. The first TOF provides high resolution precursor selection and efficient transfer of selected ions to the fragmentation region. A new pulsed accelerator following precursor selection focuses precursor ions at the second pulsed accelerator for MS-2. An RF-excited octopole is incorporated into the collision region to enhance fragment ion transmission, and a second pulsed accelerator and a second two-stage ion mirror refocus ions at the detector to provide high sensitivity, high resolution, and excellent mass accuracy for both collision-induced and unimolecular fragmentation. The ion source employs a 5 kHz laser and the pulsed accelerator operates asynchronously at speeds up to 250 times faster than conventional MALDI-TOF-TOF instruments operating at 200 Hz.

Method
The new instrument was designed using a recently developed theoretical approach for optimizing the performance of each of the major elements of a MALDI-TOF mass spectrometer. These elements include one and two-stage MALDI ion sources, two-stage ion mirrors, both gridded and gridless, time-ion selectors, ion focusing and deflecting elements, pulsed and static ion accelerators, ion fragmentors, and ion detectors. The prototype instrument was constructed to allow performance of each of these elements to be evaluated and compared with theoretical predictions. In this prototype an ion detector can be temporarily placed at any of the critical points in the ion flight path for thorough evaluation of ion beam properties and functionality of critical components. The addition of the pulsed accelerator following precursor selection allows simultaneous optimization of the performance of both MS-1 and MS-2.

Design of the System
The system comprises a two-stage pulsed MALDI source, a first two-stage gridded mirror, a Bradbury-Nielson TIS gate, a first pulsed accelerator, a field-free fragmentation chamber, a second pulsed accelerator, a second two-stage mirror and a detector. The amplitude and delay of the accelerating pulse in the ion source are adjusted to focus ions in time at a first focal point, and ions are refocused by the first ion mirror at the B-N gate. Ions selected by the gate are accelerated by the first pulsed accelerator, fragmented in the fragmentation chamber, and selected precursor ions and their fragments are accelerated by the second pulsed accelerator, separated in the second ion mirror and detected.

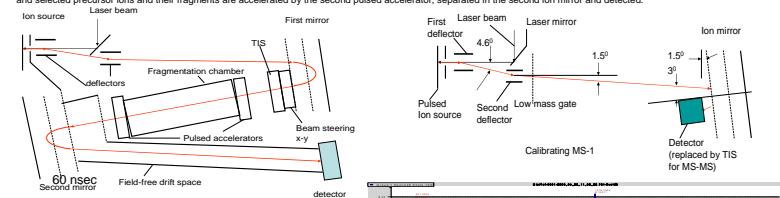


Figure 1. Schematic diagram showing layout of new TOF-TOF for high resolution precursor selection and multiplexed MS-MS measurements. Standard ion optical elements for focusing and deflecting ions are included to provide high transmission efficiency and to correct for trajectory errors¹.

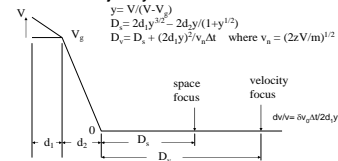
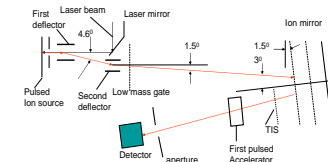
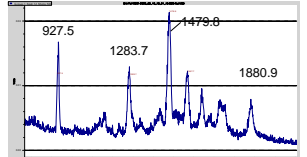


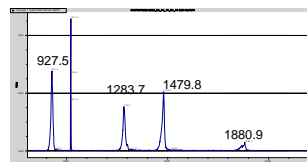
Figure 2. Potential diagram with focusing parameters and focal points for 2-stage MALDI I on source.



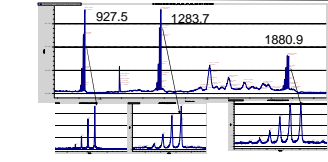
In these measurements the detector is located at the normal location for the pulsed accelerator that accelerates precursors and fragments for analysis in MS-2. Addition of the first pulsed accelerator refocuses selected ions at the second accelerator and allows high resolution performance for both MS-1 and MS-2.



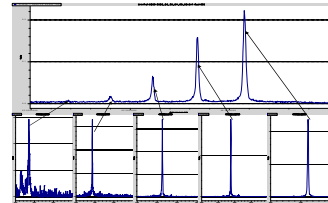
BSA digest peptides detected without selection and without velocity focusing.



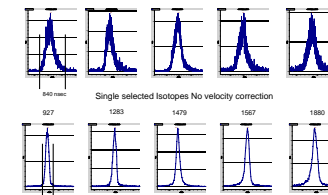
Multiplex selection of 4 major peptides from BSA digest without velocity focusing.



Multiplex velocity focusing BSA peptides without TIS selection. Note inversion of the isotope distributions. The broad peaks are masses that are not accelerated.

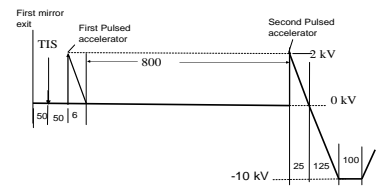


Selection of individual isotopes of 927.5 with both TIS and velocity focusing. Note selection of 931.5 with very low noise.



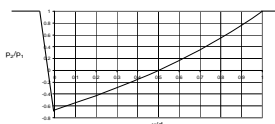
Comparison of single isotope peaks with and without velocity focusing

Theory of velocity focusing by pulsed accelerator



Potential diagram for second leg of TOF-TOF for multiplex operation with high resolution both for precursor selection in MS-1 and fragment spectra in MS-2. In the first series of experiments the detector was located at the TIS and in the second series at the second pulsed accelerator.

The velocity spread at the TIS is $p_1 = (\delta v/v)_1 = v_0 \Delta T / 2d_1 y$ and the velocity spread after acceleration in the pulse accelerator is $p_2 = (\delta v/v)_2$ where $p_2/p_1 = [V_0/V_a - D_s / 2d] / [1 + V_0/V_a]$ where d is the length of the accelerator, D_s is the distance from the TIS to the accelerator V_0 is the initial energy and V_a is the energy added by the accelerator. If p_2/p_1 is negative the velocity focusing occurs at $D_2 = D_1 - (p_1/p_2)(1 + V_0/V_a)^{1/2}$. For this case $d=6$, $D_1=50$, $D_2=800$, $V_0/V_a=0.26$ and $p_2/p_1=-0.07$. Thus the velocity spread is reduced by about a factor of 14 in good agreement with experimental results.



Calculated ratio of velocity spread vs position in the accelerator at the time the pulse is applied.

Conclusions and Future Work
Selection of single isotopes with resolving power of 4000 has been demonstrated with negligible losses in sensitivity or leakage of neighboring isotopes. The ability to select up to 10 peaks per laser shot in the mass range between 0.5 and 2.5 kDa has also been demonstrated. A new pulsed accelerator following the TIS narrows the velocity distribution at the entrance to MS-2 by more than an order of magnitude allowing high resolution measurements of fragment spectra in MS-2. The complete TOF-TOF system incorporating these advance has been assembled and is currently being tested.

References: 1. M. L. Vestal, "Modern MALDI Time of Flight Mass Spectrometry" J. Mass Spectrom. 44, 303-317(2009).
Acknowledgments
Contributions to this work from the entire staff of Virgin Instruments is gratefully acknowledged. This work was supported by the National Institutes of Health NIGMS under grant GM079832.