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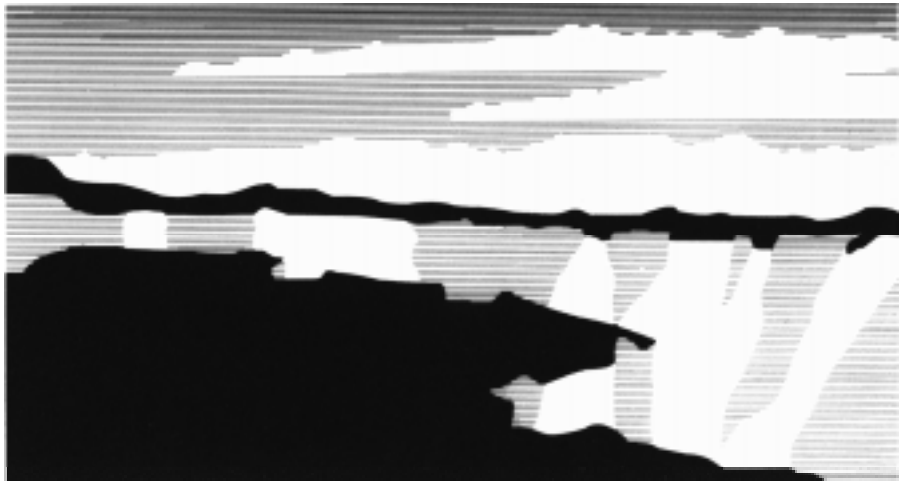
Title: **Detection and Characterization of
Biological Weapons Agents
by Matrix-Assisted Laser Desorption
Mass Spectometry**

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Los Alamos
NATIONAL LABORATORY

Detection and Characterization of Biological Weapons Agents by Matrix-Assisted Laser Desorption Mass Spectrometry

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Abstract

This is the final report of a one-year, Laboratory-Directed Research and Development (LDRD) project at the Los Alamos National Laboratory (LANL). The objective of the original, three-year, project was to develop and demonstrate a field-deployable point sensor, based on Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry, to unambiguously identify biological warfare agents. Specifically, we proposed to: (1) develop a MALDI source capable of coupling with either a reflectron time-of-fight (re-TOFMS) or ion trap (ITMS) mass spectrometer; (2) characterize the MALDI source from an instrumental and application perspective; (3) implement methodologies for biological toxin identification screening and structural verification; (4) critically compare experimental results obtained when the MALDI source is used in combination with a re-TOFMS or an ITMS instrument; and (5) design a fieldable instrument based on the detection system (re-TOFMS or ITMS) that produces the highest quality data.

Background and Research Objectives:

Biological weapons (BW) agents pose a significant and hard to detect threat to both military and civilian populations. Pathogenic effects due to BW result from either the release or production of toxins that attack body systems. Biological toxins are poisons that result from the natural metabolic processes of certain microorganisms, plants, and animals. These chemical compounds may be relatively small (e.g. alkaloids), but are often complex, high molecular weight species, such as proteins. For example, the neurotoxin Botulinum type A, with a molecular weight of approximately 150 kDa, has a greater casualty-producing potential than VX, the most lethal nerve agent. With the availability of a large number of readily producible biological toxins, and the ease with which these agents can be deployed during tactical, strategic, or terrorist operations, it is critical that sensors capable of identifying BW with high sensitivity be developed.

Technology for the rapid identification of large biomolecules has recently taken a significant step forward with the development of Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)[1, 2, 3, 4, 5]. With this technique, the biomolecule analysis is begun by mixing the sample (which can be an aerosol, liquid, or solid) with an excess of an appropriate matrix material. A small aliquot of the sample/matrix solution is then applied to a probe which is introduced into a mass spectrometer. Pulsed ultraviolet laser light is absorbed by the matrix, leading to the desorption/ablation of part of the surface. Protonation, cationization, or electron capture reactions then occur, resulting in the production of intact high molecular weight ions. The ions are separated and detected in the mass spectrometer, and the resulting data is analyzed to identify the sample.

The advantages of MALDI-MS include: 1) direct mass measurement of macromolecules up to 500 kDa; 2) speed of analysis (< 10 minutes); 3) high sensitivity (only picogram amounts of the target compound are needed); 4) simple, rugged instrumentation; and 5) the potential to identify microorganisms before they begin releasing a toxin. This last point is important because some BW agents do not become pathogenic until they are incorporated into a host (soldier or civilian). Each microorganism and/or toxin will express a unique combination (or fingerprint) of high molecular weight biomolecules which can be used to characterize the threat. In addition, some toxins require the presence of other compounds in order to express lethality. Many of these "promoters" are also high mass species that can be detected using this sensor technology. Correlation of toxin and promoter presence will provide additional confidence in the analysis.

While any type of mass spectrometer could be used for the detection of ions produced by MALDI, time-of-flight mass spectrometry (TOFMS) has historically been the method of choice for ion detection, due to the pulsed nature of the ion source and the inherent high mass capabilities of the instrument[1, 5, 6, 7, 8, 9]. However, ion trap mass spectrometry (ITMS) is also a promising detection candidate[10, 11, 12, 13, 14], and provides the ability to perform tandem mass spectrometry. The ion trap would allow for analysis of metastable ion decay products, and is more convenient for use in photofragmentation studies[15]. Because both ITMS and TOFMS have unique strengths and weaknesses, we proposed to perform a thorough comparison of detector technology to identify the most appropriate sensor system.

The objective of the our three-year project was to develop and demonstrate a field-deployable point sensor, based on MALDI-MS, to unambiguously identify biological warfare agents. Specifically, we proposed to: (1) develop a MALDI source capable of coupling with either a reflectron time-of-fight (re-TOFMS) or ion trap (ITMS) mass spectrometer; (2) characterize the MALDI source from an instrumental and application perspective; (3) implement methodologies for biological toxin identification screening and structural verification; (4) critically compare experimental results obtained when the MALDI source is used in combination with a re-TOFMS or an ITMS instrument; and (5) design a fieldable instrument based on the detection system (re-TOFMS or ITMS) that produces the highest quality data.

Importance to LANL's Science and Technology Base and National R&D Needs:

Screening for biological agents is currently on the DoD's top ten list of tough problems. This is echoed by the recent Deutch Report that recommends a significant increase in funding for the development of real-time detection technology for BW agents. Current methods are limited by poor sensitivity and slow response time. The development of MALDI-MS as a sensor system for these toxins would significantly improve the DoD's ability to protect American troops in the field. The use of MALDI-MS has been widely accepted in the biochemical community. Research in this area would, therefore, be of direct interest to pharmaceutical companies as well as the biotechnology industry.

Scientific Approach and Accomplishments:

Figure 1 schematically shows the experimental apparatus. Our work utilized a reflectron time-of-flight mass spectrometer (R. M. Jordan Co.), in combination with a pulsed Nd:YAG laser (Continuum), operating at either 355 or 266 nm. We originally attempted to use a simpler, less expensive, pulsed nitrogen laser (337 nm), but low output power and poor beam quality resulted in the production of less than threshold irradiance at the sample surface (determined by calculation). The Nd:YAG laser eliminated this problem.

A modification to the ion source of our existing instrument was necessary in order to accept MALDI samples. This was accomplished by adding a simple o-ring sealed vacuum lock to the chamber, and drilling a hole in the repeller grid of the source that would accept the high voltage probe coated with the sample. The current sample probe tips are fabricated by machining a 0.250" shoulder on a Swagelok plug to provide a smooth surface for the sample and good electrical contact to the repeller. The tip of the probe is polished to a mirror finish. Samples can be inserted from atmospheric pressure and brought to a vacuum on the order of 10^{-7} torr within two minutes.

A developmental data acquisition system was written. Data collection is triggered by a photodiode positioned to observe scattered laser light, and its signal is then adjusted via a digital delay (Stanford Research DG-535) to initiate a transient recorder (Sonix STR*864) cycle at a time corresponding to the arrival of the laser pulse at the probe tip surface. The digitized detector signal is transferred to the computer for storage and analysis. All of the calculation, control, and acquisition software was written using National Instruments' LabVIEW package.

Initial experiments were directed toward achieving measurable matrix signal and mass calibration of the instrument. The most successful matrices have been 2,5-Dihydroxybenzoic acid (DHB) and ferulic acid (trans-3-methoxy-4-hydroxycinnamic acid), but some experiments have been performed with HABA (2-(4-hydroxyphenylazo)benzoic acid), nicotinic acid, or sinapinic acid. Low mass markers were immediately found, as practically every data set contained ion signal from Na+ and K+, which likely originate in the Pyrex labware used to mix the sample solutions. The identity of all detected ions was confirmed using a flight time calculation program based on the geometry of the instrument and the applied fields. In May, we switched to using plastic beakers, flasks, etc., but we still observed these alkali metals in our spectra. It is likely that they are due to impurities in the matrices themselves. Recrystallization of the organic acids has been reported to reduce this problem, we were not able to pursue this solution. It was not difficult to observe ions due to the organic matrices. The strongest signal was almost always (M-17)+, where M is the mass of the intact matrix molecule. The loss of 17 Da corresponds to a hydroxyl (-OH) group being cleaved from the carboxylic acid moiety. Our experience, as well as that in both the academic and industrial communities, confirms that sample preparation is key to achieving signal in MALDI. The main parameters that may be adjusted in the sample preparation process are

1. matrix identity, concentration, and solvent

2. analyte identity, concentration, and solvent

3. matrix:analyte ratio

4. volume of matrix/analyte mixture applied to the probe tip

5. manner in which mixture is applied

We have investigated a significant amount of the parameter space associated with these factors. The first three are strongly interrelated. Most of the analytes need to be dissolved in aqueous solution, while many of the matrices require a small amount (5-25%) of organic

solvent (typically either ethanol or acetonitrile) in order to achieve the desired concentration. The solvent composition has a great influence on the speed of crystallization and the morphology of the deposited sample. We have found that the "best" sample preps result when the matrix solvent is, to the extent possible, aqueous. While there are no systematic guidelines for determining the optimum matrix:analyte ratio, most researchers agree that it is in the range of 500:1-10,000:1 depending on both the matrix and the analyte mass. We have used mixtures ranging from 100:1-5000:1, and find that 2000:1 is appropriate for our current measurements. Only 3 ml of sample solution is used to prepare the probe tip, which is usually good for about 30 minutes of experiments. The sample is applied to the tip using a microliter syringe, and is either allowed to dry in a static environment, or is spin-coated. The latter technique has been observed in some labs to improve probe preparation reproducibility. We have just begun to investigate the use of spin coating, so it is too early to say whether this approach is beneficial.

Our first analyte was gramicidin S (1141 Da), a cyclic peptide often used for evaluating MALDI performance. We quickly became proficient at detecting this compound in a variety of matrices (Figure 1). Bovine insulin (5733 Da) was attempted next. We initially had no success with this analyte. After a discussion with the instrument vendor, it was clear that it would not be possible to detect ions of mass 6000 or higher with the original detector. The detector was returned to the factory in mid-February to be retrofitted with a CuBe conversion dynode in front of the microchannel plate detector (MCP). High mass ions impinging on the conversion dynode more easily produce secondary electrons, which are then efficiently amplified by the MCP. Unfortunately, even with the upgraded detector, insulin detection initially evaded us. After further discussions with colleagues in industry and academia, we purchased an ion trajectory simulation package (SIMION 3-D 6.0, written by David Dahl of INEL) to model the flight of the ions in the instrument. The simulations have indicated that ion kinetic energy perpendicular to the flight tube may be a significant factor in determining the amount of signal observed, particularly for high mass species. This off-axis velocity results from the fact that the laser pulse hits the probe tip at steep angle ($\sim 80^{\text{deg}}$ with respect to the flight tube axis). Ion deflection plates, integral to the spectrometer source design, can be used to correct for this phenomenon, and we subsequently demonstrated that insulin can be detected when the appropriate voltage is applied to these plates (Figure 2).

Additional discussions, and experiments performed by the PI at Indiana University in June, 1996, suggest that perhaps the most significant cause of difficulty in obtaining high mass data is the length of the ion flight tube. A modified, 8" long tube was ordered at the end of FY96, but has not yet been tested.
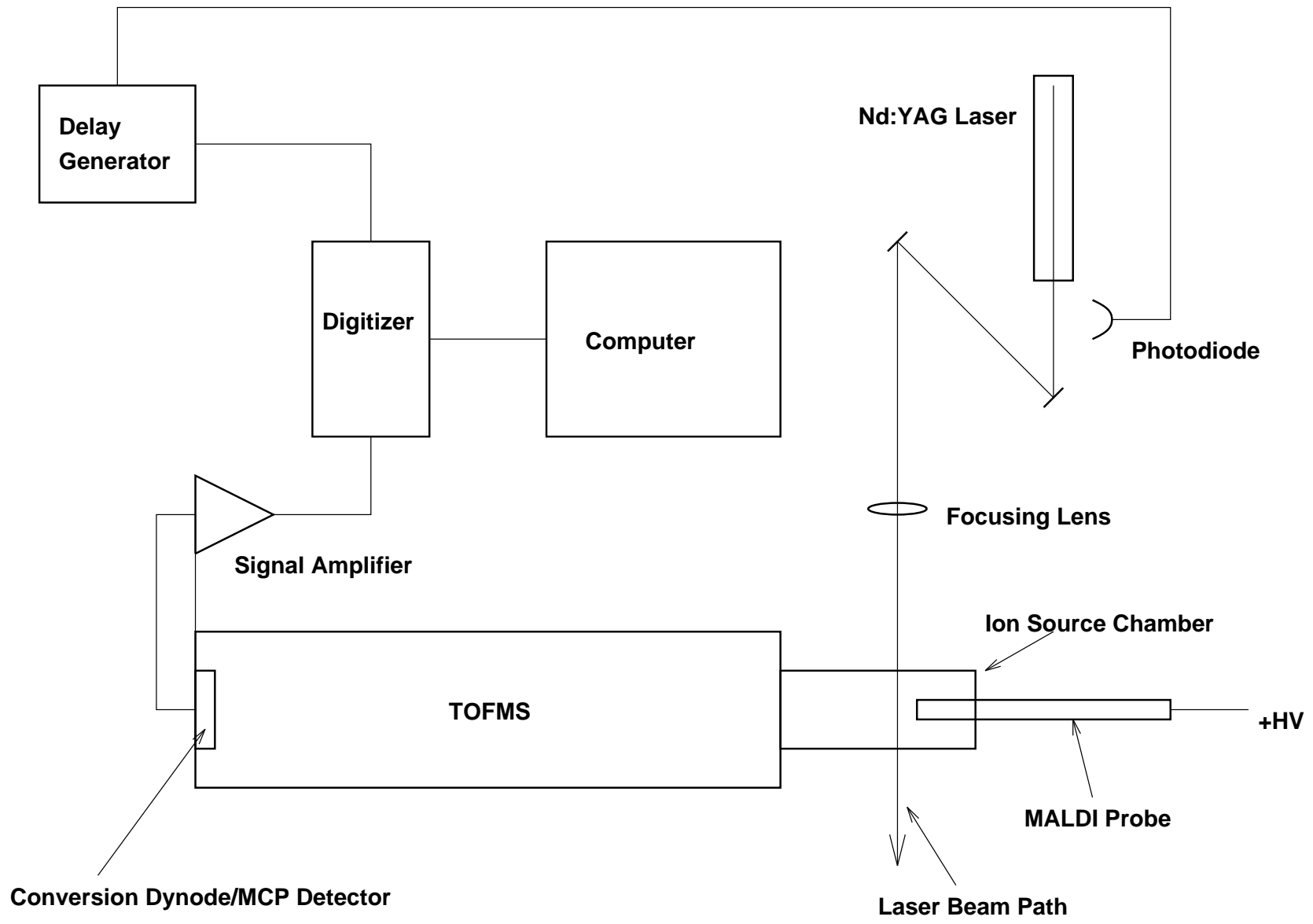
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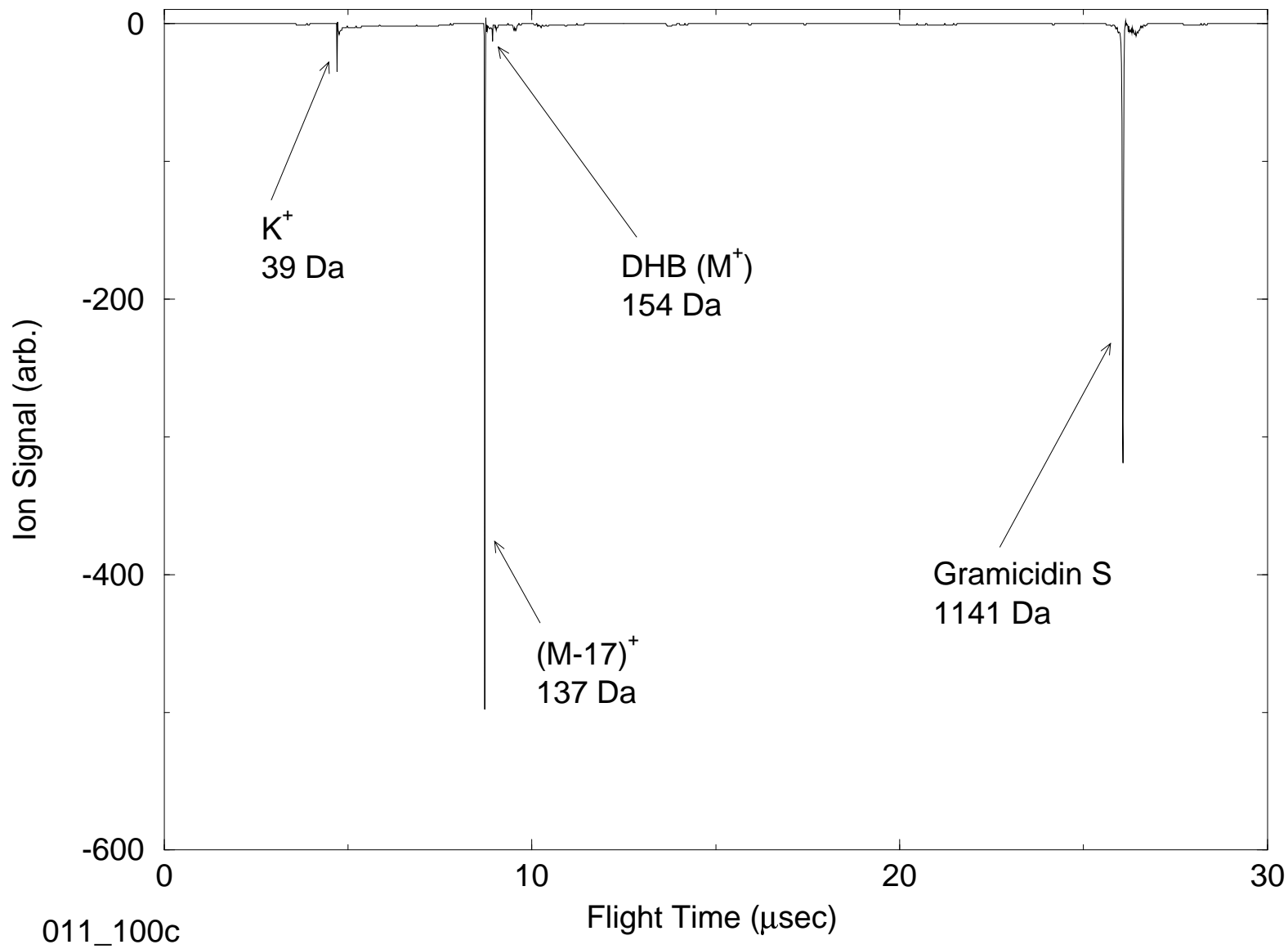[4] B. Spengler and R. Kaufmann, *Analusis*, **1992**, *20*, 91–101.

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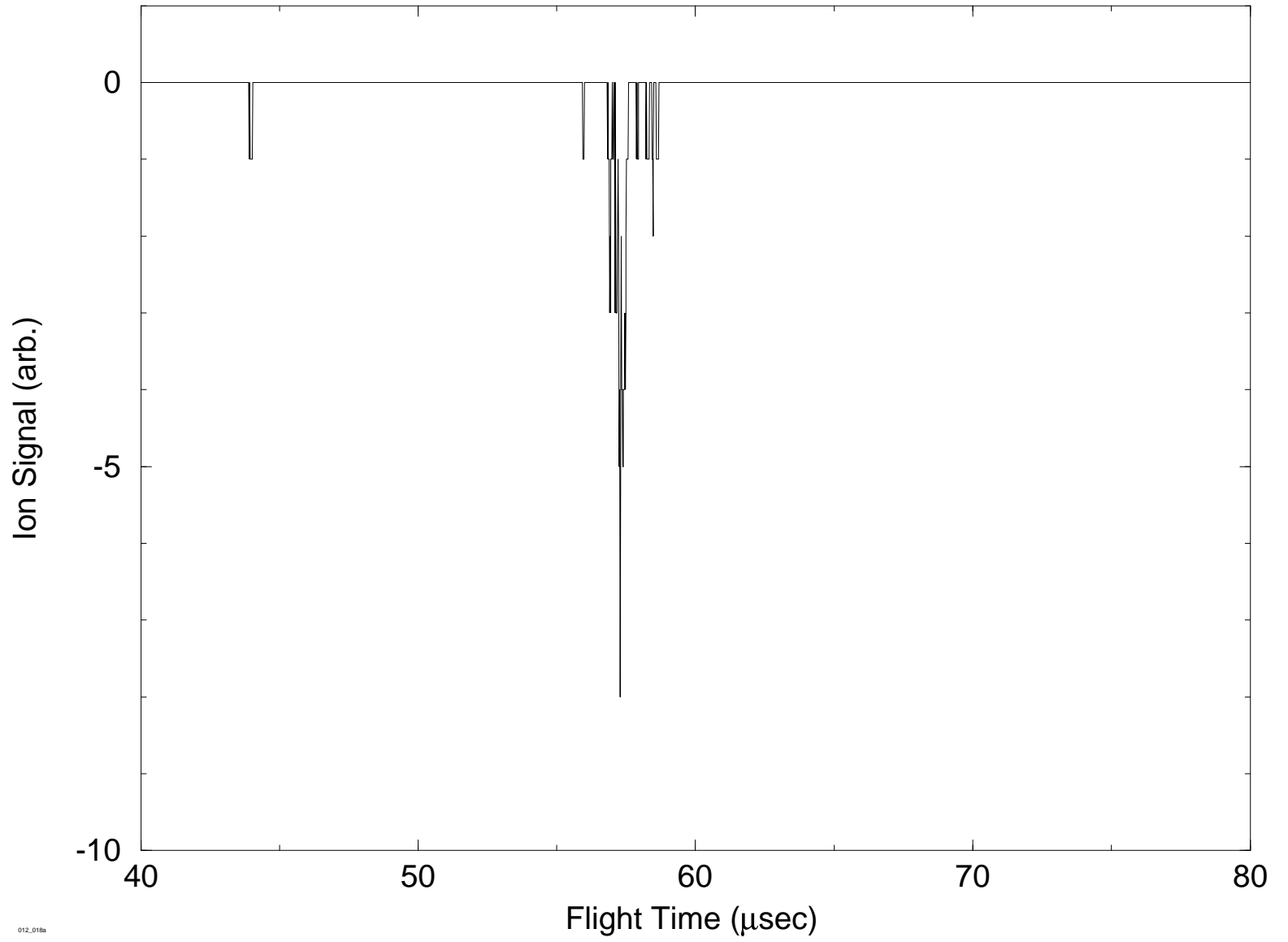[15] S. T. Fountain, H. Lee, and D. M. Lubman, *RCMS*, **1994**, *8*(5), 407–416.

Figure 1: Schematic drawing of experimental apparatus.
Figure 2: MALDI-TOFMS spectrum of 30 nmol of gramicidin S.
Figure 3: MALDI-TOFMS spectrum of parent ion region of bovine insulin.

Delay
Generator

Digitizer

Computer

Nd:YAG Laser

Photodiode

Signal Amplifier

Focusing Lens

Ion Source Chamber

TOFMS

+HV

MALDI Probe

Conversion Dynode/MCP Detector

Laser Beam Path

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