Multiple-ion-beam time-of-flight mass spectrometer

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An innovative approach to increase the throughput of mass spectrometric analyses using a multiple-ion-beam mass spectrometer is described. Two sample spots were applied onto a laser desorption/ionization target and each spot was simultaneously irradiated by a beam of quadrupled Nd:YLF laser radiation (261.75 nm) to produce ions by laser-desorption ionization. Acceleration of the ions in an electric field created parallel ion beams that were focused by two parallel einzel lens systems. After a flight path of 2.34 m, the ions were detected with a microchannel plate-phosphor screen assembly coupled with a charge coupled device camera that showed two resolved ion beams. Time-of-flight mass spectra were also obtained with this detector. Experiments were performed using both metal atom cations (Ti^+ and Cr^+) produced by laser desorption/ionization and the molecular ions of two different proteins (myoglobin and lysozyme), created by matrix assisted laser desorption/ionization using an excess of nicotinic acid as matrix. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1380682]

I. INTRODUCTION

Combinatorial approaches in areas such as drug screening,[1] discovery of new catalysts,[2,3] and the human genome project[4] have opened ways to attack important problems in chemistry, biochemistry, and materials science. Successful implementation of combinatorial methods relies on the analysis of the outcomes of many parallel experiments, for example by mass spectrometric or optical methods. Fluorescence based techniques have been successfully used to screen many experiments in parallel.[5] A more direct and general method, however, is mass spectrometry in which the mass-to-charge ratio of the analyte is directly determined. The application of mass spectrometric approaches to biochemical problems in particular have been increasing since techniques like electrospray ionization[6,7] and matrix assisted desorption and ionization (MALDI)[8–11] have become available to allow the mass spectrometric analysis of fragile biomolecules like proteins and DNA. The MALDI technique has been automated for applications like DNA sequencing[12,13] and the imaging of molecular distributions in tissue samples,[14] but commercial MALDI mass spectrometers are single-channel devices that allow the serial acquisition of the mass spectra from analyte samples. There have also been attempts to acquire spatially resolved mass spectra from targets containing many samples, but most of the work involved sequentially probing the samples with a robot-driven capillary,[15] a scanning ion beam,[16] or spatial resolution was achieved by moving the target itself.[17,18]

A significant increase in the sample throughput can be achieved if many different samples are probed simultaneously, allowing the collection of many different mass spectra per laser shot. In the present work, we have demonstrated that it is feasible to generate parallel ion beams from two different sample spots on a laser-desorption ionization (LDI) or MALDI target and simultaneously collect time-of-flight (TOF) mass spectra from these different samples. This constitutes a proof-of-principle experiment that should be scalable, allowing the development of a new generation of high-throughput TOF mass spectrometers.

II. EXPERIMENT

The setup used in this work is depicted in Fig. 1 (ion source and ion optics) and Fig. 2 (detector assembly). Ions were created by either LDI or MALDI on a stainless steel plate biased at +5 kV (for LDI) or +20 kV (for MALDI). In the LDI experiments, the plate contained two samples: titanium metal (grade 5) on the upper spot and 10 μl of an aqueous solution of CrO_3 in H_2O (subsequently dried) on the lower spot. In the MALDI experiments, the upper spot contained 5μl of 5×10^{-2} M nicotinic acid (matrix, $m/z=123.1$ u) in H_2O and 5 μl of 5×10^{-6} M myoglobin (analyte, $m/z=16950.4$ D) in H_2O. The lower spot contained 5 μl of 5×10^{-2} M nicotinic acid in H_2O and 5 μl of 5×10^{-6} M lysozyme ($m/z=14313.1$ D) in H_2O. The matrix was applied first and dried followed by the solution containing the analyte. The target spots were separated by 1 cm in both the LDI and MALDI experiments. The applied solutions were dried in a vacuum.

The quadrupled output of a Nd: YLF laser ($\lambda=261.75$ nm, $\Delta t=7$ ns, $f_{rep}=5$ Hz) was used to desorb/ionize the samples. An UV beamsplitter and mirrors were used to generate two parallel laser beams. The upper spot was irradiated with typically 9.7 μJ/ pulse, while the lower spot received typically 2.9 μJ/pulse. The two laser beams were focused by two 76 mm quartz lenses inside the vacuum chamber and hit the target plate at an angle of 20°, producing an elliptical spot with a minor axis of approximately 0.25 mm.

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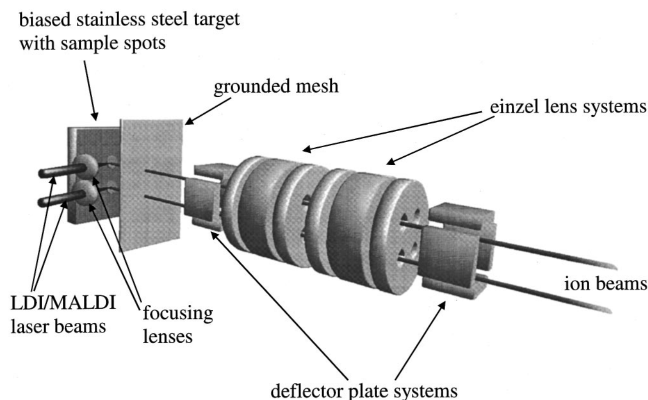


FIG. 1. Schematic of the ion optical system is shown. Ions are created by laser desorption from a biased stainless steel target and accelerated. Two multi-aperture einzel lens systems focus the ion beams and two deflector plate assemblies can be used for steering the beams.

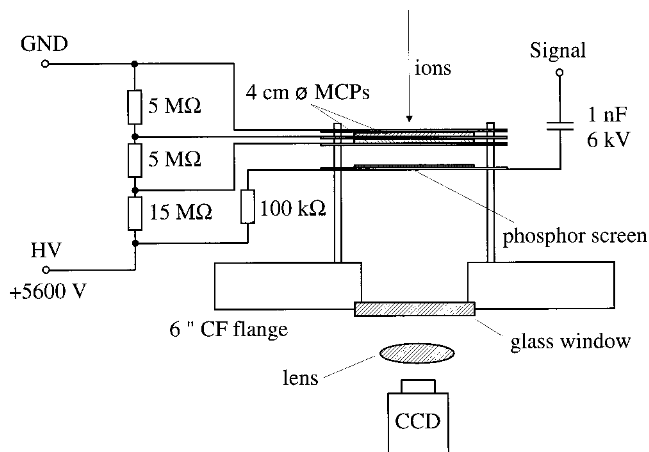
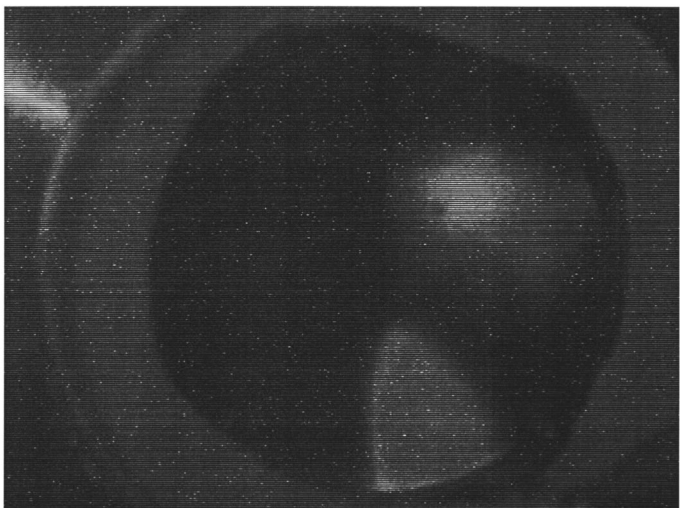


FIG. 2. Phosphor screen detector assembly is shown. Beam position information can be collected with the CCD camera and TOF signals are recorded by preamplifying the signal taken from the capacitor and feeding it into a digital storage oscilloscope.

Positive ions produced by LDI were accelerated in an electric field created by the biased target plate and a parallel, grounded wire mesh at a distance of 2 cm from the target. The ions entered an ion optics system consisting of two deflector plate assemblies and two einzel lens systems (see Fig. 1). The lens elements had four apertures of 7 mm diameter arranged in a 2×2 pattern (with a center-to-center distance of 10 mm, matching the target) to accommodate four ion beams. In the present experiments, only two of these four apertures were used to focus two parallel ion beams. The deflector plates were found to distort the ion beams so they were grounded. To suppress low-mass matrix ions in the MALDI experiments, one of the deflector plates was pulsed to +400 V for 6 μs after the laser fired to deflect low-mass ions, allowing only higher mass ions to pass through the rest of the ion optics system. For the LDI experiments ($U_{\text{target}} = +5$ kV), the first einzel lens system was set at -1590 V, the second one was at 0 V. For $U_{\text{target}} = +20$ kV (MALDI experiments) the first einzel lens system was operated at -5600 V, the second one was again at 0 V.

After a flight path of 2.34 m the ions impinged on a chevron microchannel plate/phosphor screen detector assembly (depicted in Fig. 2). The MCPs were 4 cm in diameter (Photonis G12/46-DT13), and the phosphor screen (ZnS:Ag, P-22 blue) had a diameter of 3.7 cm. The ZnS:Ag layer was deposited onto a conducting (indium–tin oxide) glass plate for use as a TOF recording anode. The detected ion signal from the phosphor screen was amplified by a fast preamplifier (home built using a Comlinear CLC400 fast operational amplifier) and fed into a digital storage oscilloscope (Tektronix TDS3052) for recording TOF spectra. A LABVIEW program was used to record, accumulate, and calibrate the TOF mass spectra. The spatial extent of the separate ion beams was recorded by collecting the visible light emission from the phosphor screen anode with a scientific grade CCD/ camera (Cohu 4910) operating in time-integration mode, i.e., the camera had its electronic shutter open for typically 4 s and the accumulated image was sent to a frame grabber card (Data Translation DT3155) in a PC for further processing. The pressure inside the apparatus was typically 10^{-7} Torr inside the source region (pumped by a diffusion pump) and 10^{-8} Torr inside the detector region (pumped by two turbomolecular pumps).



3.7 cm

FIG. 3. CCD image showing two distinct bright spots corresponding to two different sample spots (Ti, upper spot, and CrO_3, lower spot) on the target.

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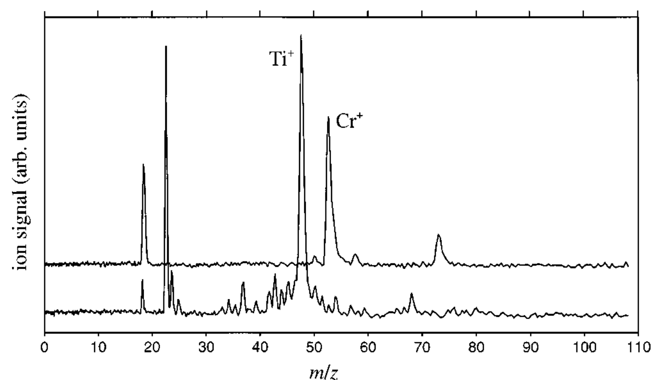

FIG. 4. Mass spectra recorded with two different sample spots (Ti and CrO_3) are shown.

III. RESULTS

Figure 3 is an image of the phosphor screen recorded with the charge coupled device (CCD) camera that shows two distinct bright spots caused by the two parallel ion beams. The upper beam was created from titanium on the upper target spot, the lower beam originated from a dried spot of CrO_3 solution on the lower target spot. The ions were created by LDI as no matrix was present. The most intense parts of the two spots on the phosphor screen are separated by ≈20 mm. The upper spot is approximately circular with a diameter of 7 mm, while the lower spot is a truncated oval with dimensions approximately 10×13 mm. In any case, the beams are not overlapped or crossed, i.e., the upper emission spot corresponds to the upper sample on the target. The beams are not entirely parallel, however, as the spacing has increased from 10 to 20 mm over the 2.34 m flight path. Installation of an array of ion beam deflectors should allow correction for this small angular deviation, which amounts to ≈0.25° over this distance. TOF mass spectra were obtained during the same experiment by feeding the electrical signal from the phosphor screen (acting as anode) into a preamplifier and storage oscilloscope. Figure 4 shows the mass spectra of Ti^+ (from the upper target spot) and Cr^+ (from the lower target spot). The spectra were taken in succession by

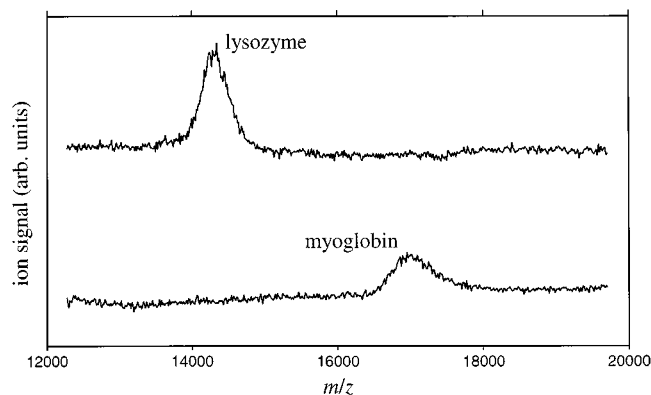

FIG. 5. Mass spectra recorded from two different proteins (myoglobin, $m/z = 16\,950.4$ D, upper spot, and lysozyme, $m/z = 14\,313.1$ D, lower spot) are shown.

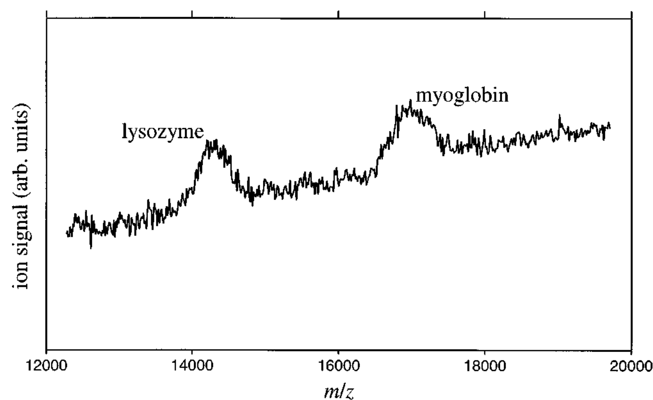

FIG. 6. Mass spectra of the two different proteins recorded simultaneously using two ion beams are shown.

blocking one of the laser beams and passing the other to the appropriate target spot.

MALDI experiments were done on myoglobin (applied to the upper target spot) and lysozyme (on the lower spot) in a large (10000:1) excess of matrix. Figure 5 shows two protein TOF mass spectra taken in succession (one MALDI laser beam was blocked, while the other hit the appropriate target), while Fig. 6 shows a spectrum which was taken by passing both laser beams to the target spots and allowing both ion beams to hit the detector simultaneously. With the CCD detector, it was evident that the two protein ion beams did not interfere, as shown for the metal cations in Fig. 3. The resolution in the mass spectra is rather low because no attempts (i.e., use of Wiley–McLaren two-field extraction[19] or delayed extraction techniques[20,21]) were made to increase the resolution in these proof-of-principle experiments. In any case, however, the relatively large kinetic energies the massive protein cations reach during the desorption/ionization step in the presence of large quantities of lighter-mass matrix particles does not prevent successful operation of a multiple-ion-beam mass spectrometer.

IV. DISCUSSION

In this study, we have shown that it is possible to generate parallel ion beams from distinct sample spots on a LDI/MALDI target illuminated by a laser beam for each spot. A multi-aperture ion lens system enabled us to focus each ion beam individually and image two beams 2.34 m away from the target on a phosphor screen detector. In addition, it was demonstrated that each ion beam can be used to obtain mass spectrometric information on the individual sample spots. This procedure worked well for low-mass ions (Cr^+ and Ti^+) as well as for heavier protein ions ($m/z = 14\,313.1$ D and 16 950.4 D).

In future efforts, it should be straightforward to extend this method to more parallel ion beams, e.g., arranged in a $n \times m$ array, enabling the measurement of mass spectra of different analytes simultaneously in one experiment and thus increasing the sample-throughput considerably. In order to do that, it will be necessary to design an optical system that creates and focuses many parallel laser beams. One way to achieve this is expanding a beam from an UV laser with

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enough power to irradiate $n \times m$ sample spots and using a multi-element $n \times m$ lens array for focusing. The phosphor screen in the detector assembly will have to be replaced by an arrangement of $n \times m$ anode plates, each one connected to a fast preamplifier and transient recorder. Each anode will then record its own mass spectrum from the corresponding sample spot on the target plate. The fidelity of ion beam transport will need to be improved to allow a higher density of analyte spots with the current 4 cm diameter detector. Insertion of an array of ion beam deflectors and additional focusing elements will make this possible. True high-throughput operation of the spectrometer will also of course entail construction of a sample-handling system allowing for rapid exchange of targets. On-line calibration of the spectrometer in high-throughput analysis configurations should be achievable by interspersing calibration samples with unknown samples on the target arrays.

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[1] C. E. Barry, R. A. Slayden, A. E. Sampson, and R. E. Lee, Biochem. Pharmacol. **59**, 221 (2000); L. Gold and J. Alper, Nat. Biotechnol. **15**, 297 (1997).
[2] B. Jandeleit, D. J. Schaefer, T. S. Powers, H. W. Turner, and W. H. Weinberg, Angew. Chem. Int. Ed. Engl. **38**, 2495 (1999).
[3] S. Senkan, Nature (London) **394**, 350 (1998).
[4] P. O. Brown and D. Botstein, Nature Genetics Suppl. **21**, 33 (1999).
[5] N. S. Finney, Curr. Opin. Drug. Disc. Dev. **1**, 98 (1998).
[6] *Electrospray Ionization Mass Spectrometry*, edited by R. B. Cole (Wiley, New York, 1997).
[7] C. Hinderling and P. Chen, Angew. Chem. Int. Ed. Engl. **38**, 2253 (1999).
[8] M. Karas, D. Bachmann, U. Bahr, and F. Hillenkamp, Int. J. Mass Spectrom. Ion Processes **78**, 53 (1987).
[9] M. Karas and F. Hillenkamp, Anal. Chem. **60**, 2299 (1988).
[10] B. Spengler and R. J. Cotter, Anal. Chem. **62**, 793 (1990).
[11] M. Karas, U. Bahr, and U. Giessmann, Mass Spectrom. Rev. **10**, 335 (1991).
[12] P. Ross, L. Hall, I. Smirnov, and L. Haff, Nat. Biotechnol. **16**, 1347 (1998).
[13] P. L. Ross, K. Lee, and P. Belgrader, Anal. Chem. **69**, 4197 (1997).
[14] R. M. Caprioli, T. B. Farmer, and J. Gile, Anal. Chem. **69**, 4751 (1997).
[15] M. Orschel, J. Klein, H.-W. Schmidt, and W. F. Maier, Angew. Chem. Int. Ed. Engl. **38**, 2791 (1999).
[16] R. M. Braun, A. Beyder, J. Y. Xu, M. C. Wood, A. G. Ewing, and N. Winograd, Anal. Chem. **71**, 3318 (1999).
[17] S. M. Hankin, P. John, A. W. Simpson, and G. P. Smith, Anal. Chem. **68**, 3238 (1996).
[18] D. P. Little, T. J. Cornish, M. J. O'Donnell, A. Braun, R. J. Cotter, and H. Köster, Anal. Chem. **69**, 4540 (1997).
[19] W. C. Wiley and I. H. McLaren, Rev. Sci. Instrum. **26**, 1150 (1955).
[20] R. M. Whittal and L. Li, Anal. Chem. **67**, 1950 (1995).
[21] R. D. Edmondson and D. H. Russell, J. Am. Soc. Mass Spectrom. **7**, 995 (1996).

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