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應用手冊

MALDI-Tof Using an Erbium-Yag Infra-Red Laser and Time-Lag-Focusing

Bateman, R., Jeffery M. Brown, Williams, T., Kosaka, T., Kinoshita, T., Nakamura, T.

Waters Corporation, Jasco International Ltd., Sankyo Co. Ltd

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Abstract

Initial data from a MALDI-Tof mass spectrometer using an Infra-Red (IR) laser is presented.

Despite the relatively long duration of the IR laser pulse, the time-lag-focusing (TLF) source provides relatively high resolution (4000, FWHM) and good mass accuracy for peptides.

IR laser MALDI-Tof systems have the potential to provide data directly from electroblots and electrophoresis gels.

Benefits

Good mass measurement accuracy and resolution has been demonstrated for peptides using an infra-red laser on a MALDI-Tof mass spectrometer using time lag focusing despite the long duration pulse of the laser.

Introduction

Matrix-assisted laser desorption ionization time of flight mass spectrometers usually incorporate ultra-violet (UV) laser systems as they are capable of ionizing many classes of biochemical and synthetic polymers. They provide short pulse widths, are reliable and relatively inexpensive.

In contrast IR lasers have been considered less suitable for MALDI-Tof instruments because of their long laser pulse duration and relatively large variations in output energy from shot to shot.

Despite this, there has been some interest in the use of IR lasers, for direct ionization of proteins from electroblots.[1]

Initial results obtained for peptides when using an IR laser in conjunction with a TLF source on a Waters Micromass TofSpec-SE are presented. Resolving powers in excess of 4000 (FWHM) for renin substrate (MH^+ 1758.933) are observed, despite the very long pulse duration of the IR laser.

Experimental

The data presented was acquired on a TofSpec-SE time of flight instrument fitted with Time Lag Focusing.[2] A schematic of the geometry of the instrument is shown in Figure 1.

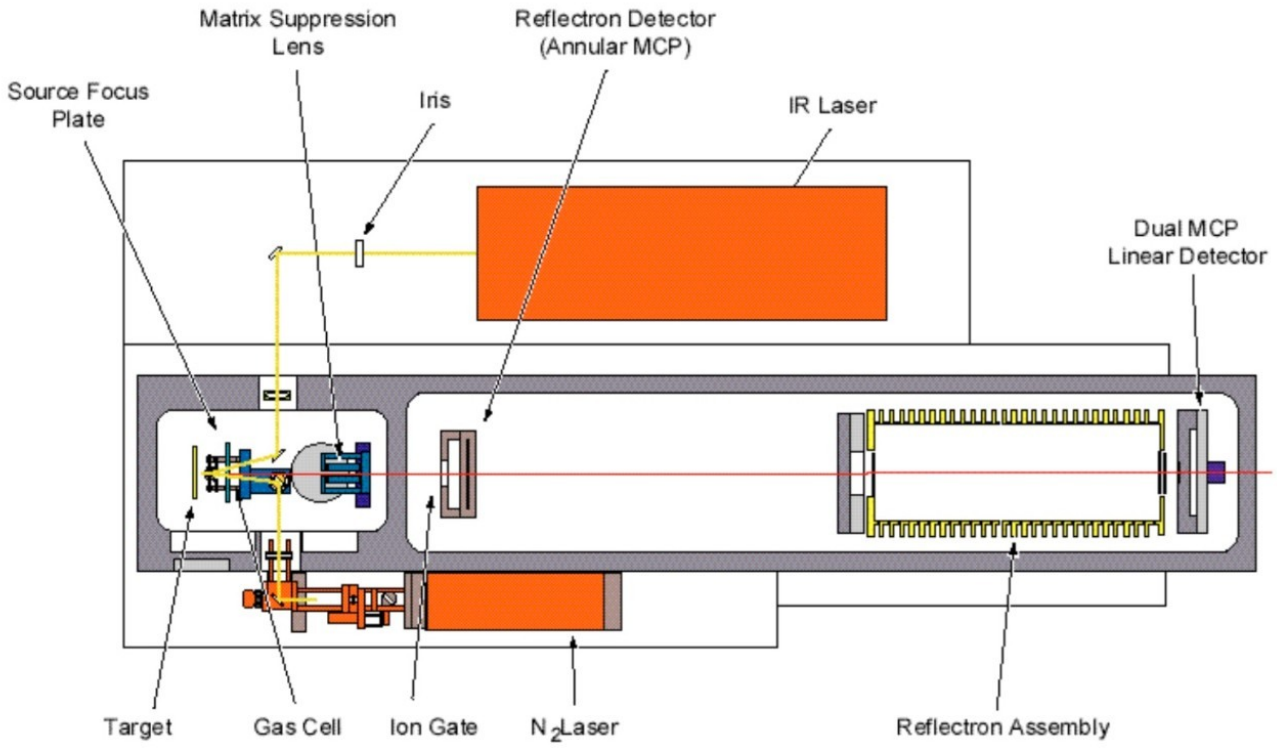


Figure 1. Schematic of instrument.

The two-stage ion source operates at up to 30kV and the instrument has an effective path length of 3.5 meters in reflectron mode.

The reflectron detector is an Hamamatsu dual microchannel plate.

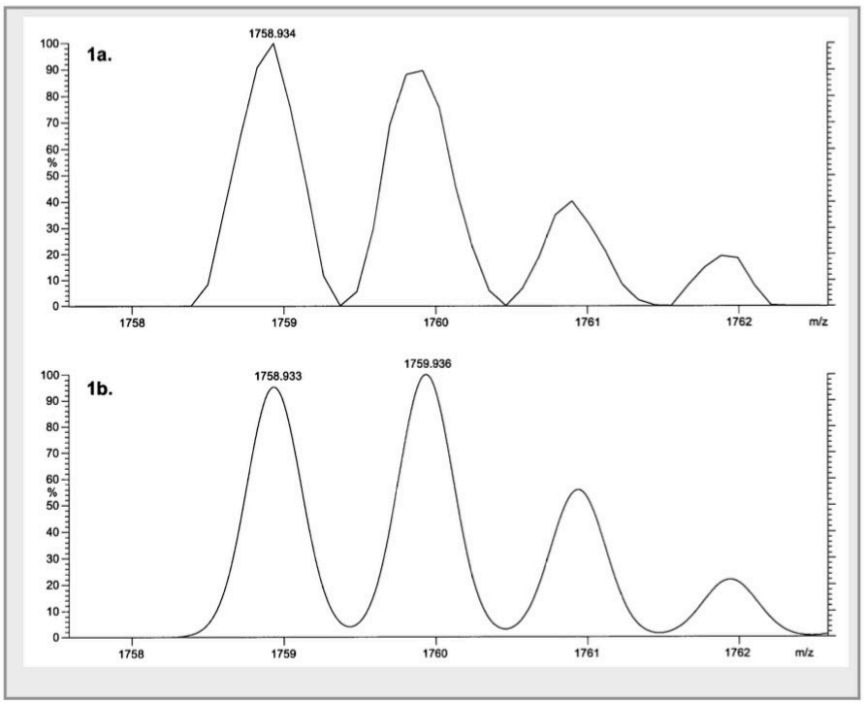
Data were acquired using a 500 Mhz, 8-bit transient recorder.

Laser Systems

Both UV and IR laser light ports are available for irradiation of the sample.

The UV laser is a Laser Science Inc. (337 nm) UV laser with pulse widths of 3 nsecs (FWHM) and attenuation is controlled by a combination of a stepped neutral density filter and an iris. The IR laser is a Schwartz Inc. solid state, rotary Q-switched Erbium-Yag System (2940 nm) and attenuation is achieved by control of the laser flashlamp voltage and an iris. The energy of the laser is monitored by a “Coherent Inc.” power meter. The

pulse widths are approximately 300 nanosecs.



Spectrum 1a and 1b Spectrum of Renin Substrate using IR laser.

Resolution of 4000 FWHM for Renin Substrate using IR laser

Spectrum 1a shows Renin Substrate (MH^+ 1758.933) acquired in reflectron mode using the IR laser. Spectrum 1b shows a theoretical spectrum of Renin Substrate resolved to 4000 (FWHM) for comparison with Spectrum 1a. The matrix used was succinic acid (10 mg/ml, dissolved in CH_3CN/H_2O=1/1). 100 picomoles of sample was loaded onto the target.

Mass measurement accuracy for Renin Substrate using the IR laser

Table 1 shows the mass accuracy obtained for Renin substrate from 11 separate spectra. The spectra were calibrated using Gramicidin S (MH^+ 1141.714) and ACTH 18-39 (MH^+ 2465.199) with the UV laser, from a separate sample spot.

Observed mass (MH+)	Mass error (ppm)	Error summary (ppm)	
1758.86	-42	Mean	9
1759.11	101		
1758.95	10	Stan. dev.	54
1758.92	-7		
1759.02	49	RMS	53
1758.97	21		
1759.1	95		
1758.84	-53		
1758.83	-59		
1758.9	-19		
1758.93	-2		

Table 1. Mass measurement accuracy for renin substrate using the IR laser.

Results and Discussion

In the TLF mode with the standard short pulse UV laser, after the laser pulse, ions are desorbed and disperse in the field free region of the source according to their initial velocities. Ions of a particular velocity expand to a particular point in space. This is known as space velocity correlation and is depicted graphically in Figure 2.

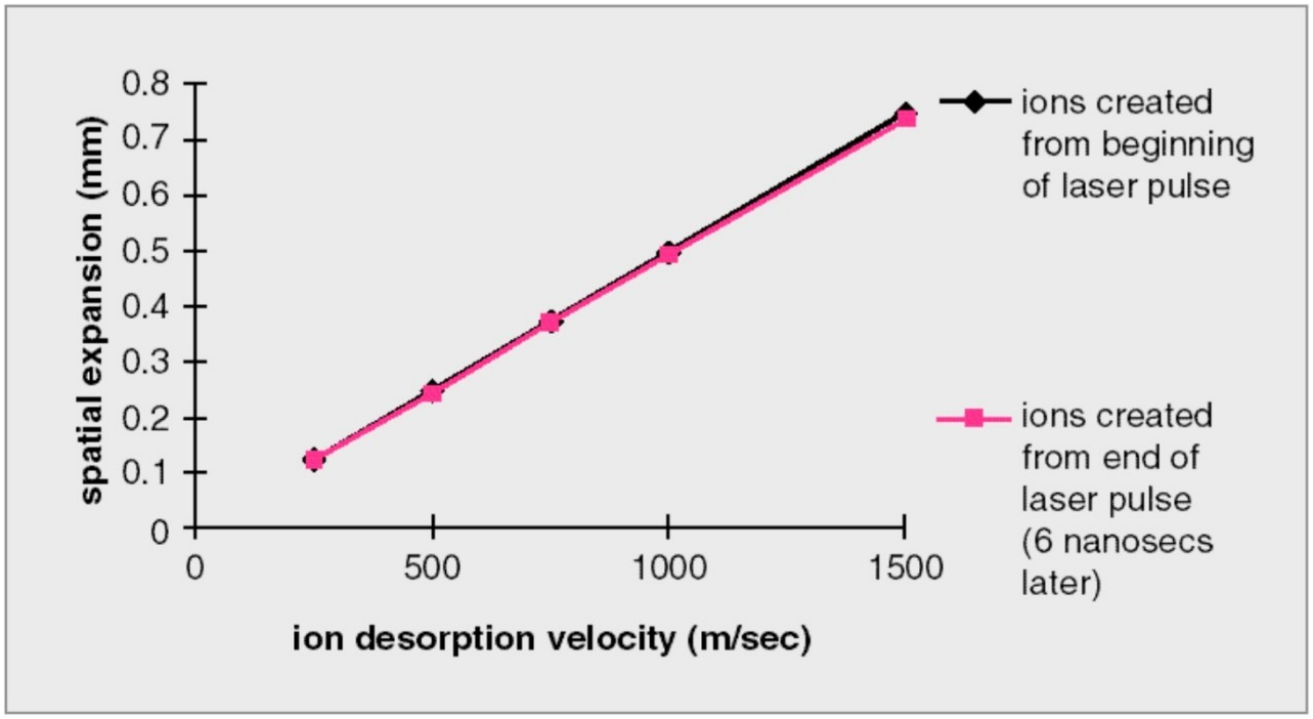


Figure 2. Space-velocity-correlation with short pulse (3 nsecs) UV laser, 500 nsecs after pulse.

After an appropriate delay time an electrical extraction field is applied to the ions such that they are brought into temporal focus at the detector. However, with the IR laser, the laser pulse is several hundred nanoseconds long and the space velocity correlation effect is blurred. This can be seen graphically in Figure 3 where ions of differing velocities have expanded to the same point in space as a result of the duration of the ionizing laser pulse.

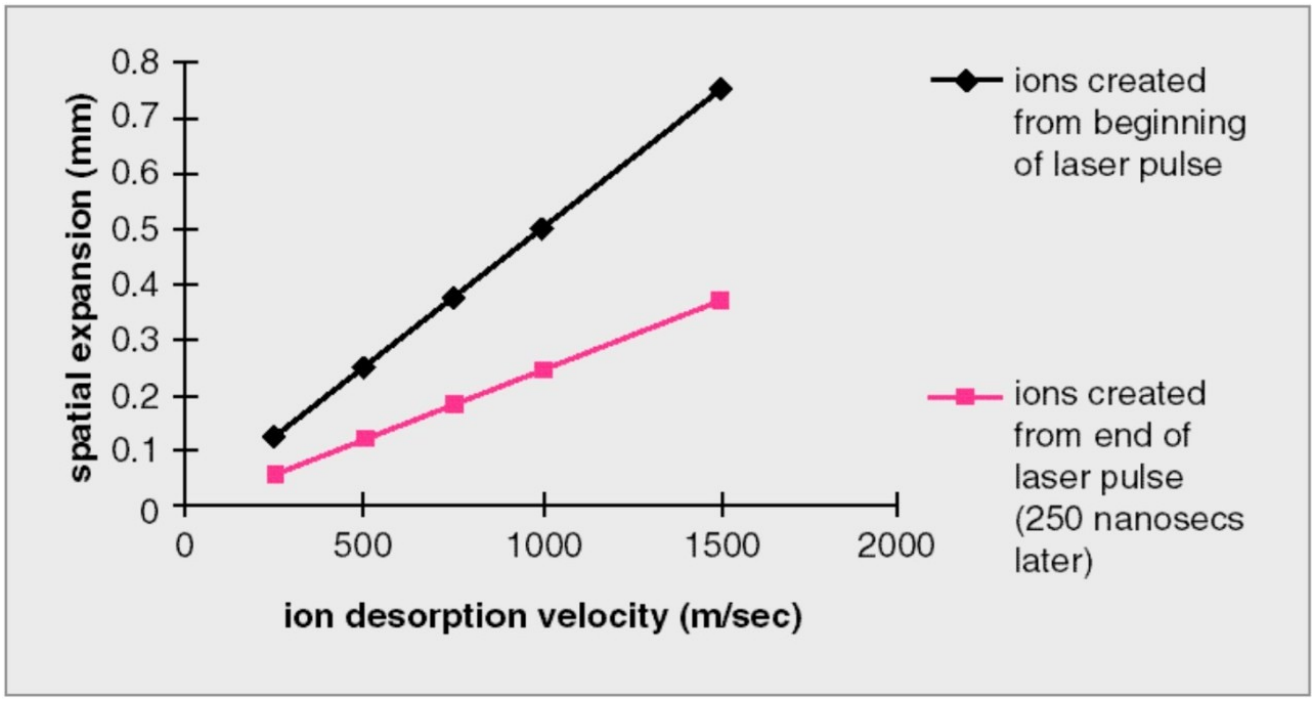


Figure 3. Space-velocity-correlation with long pulse (250 nsecs) IR laser, 500 nsecs after pulse.

Despite this blurring, it is still possible to set a combination of pulse delay time and extraction field such that ions are time focused at the detector as shown in spectrum 1a.

Conclusion

Good mass measurement accuracy and resolution has been demonstrated for peptides using an infra red laser on a MALDI-Tof mass spectrometer using time lag focusing despite the long duration pulse of the laser.

References

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2. Wiley W. C. and McLaren I. H., *Rev. Sci. Instrum.* 26, 1150 (1955).

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