Low Attomole Sensitivity with Optimised MALDI ToF Zoom Optics

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Overview

- In this paper we describe an improved MALDI Time of Flight Mass Spectrometer wherein the laser fluence is controlled by varying the area of illumination in conjunction with the beam intensity.
- The zoom laser optics enables over an order of magnitude of variation in the amount of sample irradiated and also has the added benefit of a uniform 'top hat' profile so that the entire irradiated sample experiences the same fluence. Coupling this to the matched zoom ion optics shows an improvement in useful sensitivity of greater than an order of magnitude.
- Low attomole sensitivity has been routinely achieved for a mixture of peptides.

Introduction

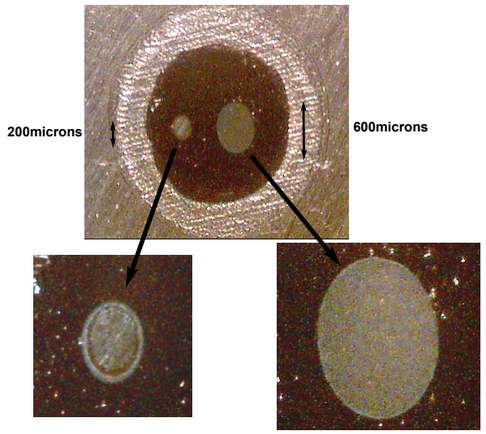
- One of the major factors limiting MALDI sensitivity is the uneven laser fluence on the sample spot produced by an, at best, Gaussian laser beam.
- A more homogenous laser spot produces a much greater number of collectable ions leading to an increase in sensitivity.
- By producing a more homogenous laser spot much larger laser spot sizes can also be used.
- A larger laser spot size reduces the problem of inhomogeneous sample deposition and reduces the 'hunting time' for a good area of sample leading to a much higher sensitivity and shorter analysis time.

Method

The Laser Optical System

- The laser optical system uses an optical fibre to introduce multiple reflections and give a homogeneous laser fluence over the entire laser spot. This is then coupled into a zoom kernel that allows variation in the diameter of the laser spot from 50 microns to 800 microns onto the sample, which gives a more precise laser fluence control.

Images of the Laser Spot Shape

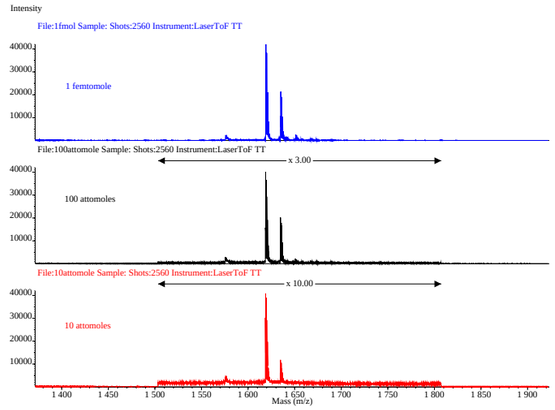


The Ion Optical System

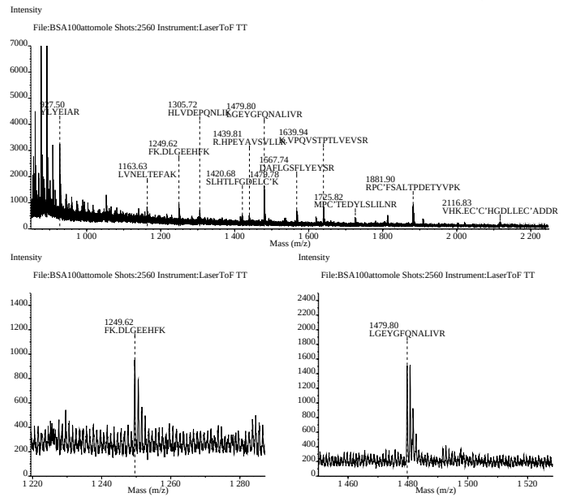
- The laser zoom optics system is coupled to a Variable Field of View Time of Flight Instrument, which is ion optically matched to the size of the laser spot. This consists of a two lens ion optical system coupled to an ideal field reflectron thus enabling high resolution and mass accuracy ToF MS and TOF TOF MS/MS data.
  For a more complete description of the Laser and Ion Optical system see Reference 1.

Results

- Dilution series of a single peptide (Bombesin m/z1620) and of BSA digest were tested.
- Bombesin was clearly detected down to 10attomoles on sample.



- The dilution series of BSA digest gave a correct identification down to 100attomoles on sample.



- **Database Search Results at 100 attomoles Concentration**

ALBU_BOVIN
Fixed modifications: Carboxymethyl (C)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: 13
Number of mass values matched: 12
Sequence Coverage: 24%

Start - End	Observed	Mr(expt)	Mr(calc)	Delta	Miss	Sequence
35 - 44	1249.6158	1248.6086	1248.6138	-0.0053	1	R.FKDLGEEHFK.G
66 - 75	1163.6142	1162.6069	1162.6233	-0.0165	0	K.LVNELTEFAK.T
89 - 100	1420.6616	1419.6544	1419.6704	-0.0160	0	K.SLHTLFGDELCK.V
161 - 167	927.5175	926.5102	926.4861	0.0241	0	K.YLYEIAR.R
264 - 280	2116.8612	2115.8539	2115.8295	0.0244	1	K.VHKECCHGDLLECADDR.A
347 - 359	1567.7201	1566.7128	1566.7354	-0.0226	0	K.DAFLGSFLYEYSR.R
360 - 371	1439.7933	1438.7860	1438.8044	-0.0184	1	R.RHPEYAVSVLLR.L
402 - 412	1305.6765	1304.6693	1304.7088	-0.0395	0	K.HLVDEPQNLIK.Q
421 - 433	1479.7833	1478.7761	1478.7881	-0.0120	0	K.LGEYGFQNALIVR.Y
437 - 451	1639.9276	1638.9203	1638.9304	-0.0101	1	R.KVPQVSTPTLVEVSR.S
469 - 482	1725.7872	1724.7800	1724.8113	-0.0314	0	R.MPCTEDYLSLILNR.L
508 - 523	1881.9017	1880.8945	1880.8978	-0.0034	0	R.RPCFSALTPDETYVPK.A

RMS Error 15ppm External Calibration

- **Sample Preparation**

Bombesin Dilutions. Bombesin (Sigma) was serially diluted in 0.1% trifluoracetic acid from a stock of 10 picomole /µl to 10 attomole /µl.
BSA Digest Dilution. BSA digest (Michrom, PTD/00001/15) was serially diluted 1:10 from a stock of 100 femtomole /µl to 100 attomole / µl.
Samples were co crystallized onto a steel target with 2.5 mg/ml α-cyano-4-hyroxy-cinnamic acid in solvent, 0.1% trifluoroacetic acid and 50% aqueous acetonitrile.

Conclusion

- The use of the Zoom Optics gives over an order of magnitude improvement in useful sensitivity compared to conventional optics.
- The data shows 10attomoles on sample of a single peptide are achievable.
- The digested BSA data shows that 100 attomoles sensitivity can give a conclusive mass fingerprint. The data shows that the sensitivity is now limited by the chemical noise.

References

1. M Mills et al, Zoom Optics for Optimised MALDI Sensitivity, ASMS Poster 2005