Proceedings of the Technical Sessions, 26 (2010) 41-47
Institute of Physics – Sri Lanka

Study of the laser spot size on the surface of a MALDI target with the focusing height in imaging mass spectrometry

Vijanaka Fernando[1], Werner Ens[2], Victor Spicer[2], Hui Qiao[2] and T.R. Ariyaratne[3]
[1]*Department of Physics, The Open University of Sri Lanka, Nawala*
[2]*Department of Physics and Astronomy, University of Manitoba, Winnipeg, Canada*
[3]*Deprtment of Physics, University of Colombo, Colombo 03*

ABSTRACT

Matrix Assisted Laser Desorption/Ionization (MALDI) imaging mass spectrometry is performed by ablating the entire sample surface with laser spots in a pattern of array. The laser spot size, focused on the sample surface is a major parameter in determining the spatial resolution of MALDI imaging mass spectrometry. The variation of laser spot size on the MALDI target with different focusing heights was studied in this research. The burnt marks on a thin layer of 4-hydroxy-α-cyanocinnamic acid (α-HCCA) produced by the N_2 laser coupled to the optical system were compared by analysing their optical images using *Digimizer*^{TM} (trial version) software. According to the results, the laser spot size on the MALDI target changes about 15 % from the beam waist, within the focusing height of 200 μm.

1. INTRODUCTION

Mass spectrometer is an analytical instrument that converts neutral molecules into gaseous ions and separates them ions according to their mass to charge ratio (m/z). With the introduction of ionization techniques of MALDI [1,2], mass spectrometry has revolutionized the investigation of biological molecules by providing soft ionization. In the analysis of complex samples such as biological tissues, MALDI is exceptional because of its ability to desorb and ionize molecules of high weight, such as proteins and peptides. In addition to that this ionization technique provides excellent sensitivity while retaining considerable tolerance towards salt and other small molecules found at high concentration in biological tissues.

Molecular imaging mass spectrometry is used to reveal the relative distribution of peptides and proteins throughout a sample surface (biological tissue). Recording the distribution of elements and bio-molecules in cells and tissues will help to address many issues in today's cell biology and medicine. MALDI mass spectrometry has been used for imaging purposes a decade ago to create chemical images of substrates [3,4]. Caprioli and coworkers [5,6] were largely involved in developing techniques and instrumentation for MALDI imaging mass spectrometry.

The MALDI imaging mass spectrometry is performed producing the molecular image of the sample surface using the position correlated mass spectral data. The

Figure 1 shows the ablation of a mouse brain tissue section using a laser microprobe and an ion detector in the conventional MALDI imaging mass spectrometer.

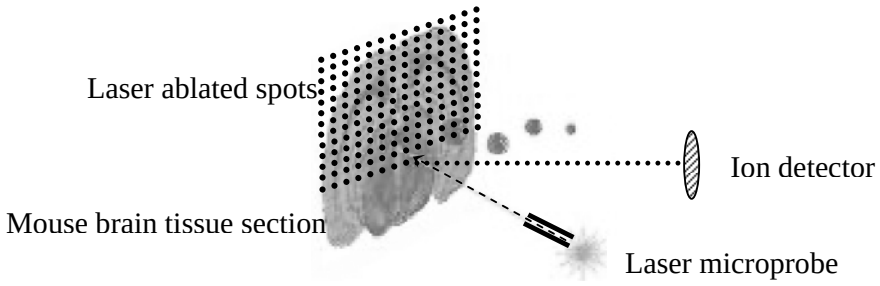

Figure 1: MALDI imaging mass spectrometry technique

In principle, the mass spectral molecular image represents more accurately the real specimen, when the sampling area is as small as possible. In conventional MALDI imaging mass spectrometry, this can be achieved by scanning the specimen area with smaller sites using a laser microprobe. The pixel size of the image or the spot size of the ionization beam is typically referred to as the spatial resolution in MALDI imaging mass spectrometry. The molecular image of a biological tissue sample with higher spatial resolution helps to uniquely identify the real location of the interested area of the scanned specimen. Therefore it is important to have a smaller laser spot on the MALDI target to produce a quality molecular image of an object in imaging mass spectrometry.

In most mass spectrometers, the laser microprobe consists of lenses to expand and focus the beam and optical fibres to deliver the laser on to the MALDI target. The optical properties such as laser divergence angle, diffraction and spherical aberration which effect on the laser spot size were clearly defined and experimentally proved in the past. And also a number of instrumental designs, reducing the laser spot size have been reported in the field of mass spectrometry [7-10].

The small spot size of a highly focused laser significantly reduces the ion sensitivity and mass resolution in axial MALDI ToF (Time-of-Flight) investigation. This situation is somewhat different in the decoupled ion source of orthogonal-injection mass spectrometers. The effect of laser profile, fluence and spot size on the ion sensitivity in orthogonal-injection MALDI TOF mass spectrometers were studied by Werner et al [11] recently. According to their investigation, the optimum sensitivity was achieved at a high fluence and the highest integrated yield per unit area was achieved with the smallest spot size. This paper investigates the laser spot size on the surface of a MALDI target with the focusing height to achieve the smallest spot size for in imaging mass spectrometry.

2. EXPERIMENTAL

A 30 mg of α-HCCA matrix dissolved in 1 ml of pure acetone, mixed with a drop of dye and a 5µl of the solution was laid on the target to prepare a thin layer of α-HCCA material. This sample was used to study the size and shape of the laser spot on the sample plate.

Figure 2 shows the optical arrangement which was used to record the laser spot area on the thin layer of α-HCCA deposited on a metal plate.

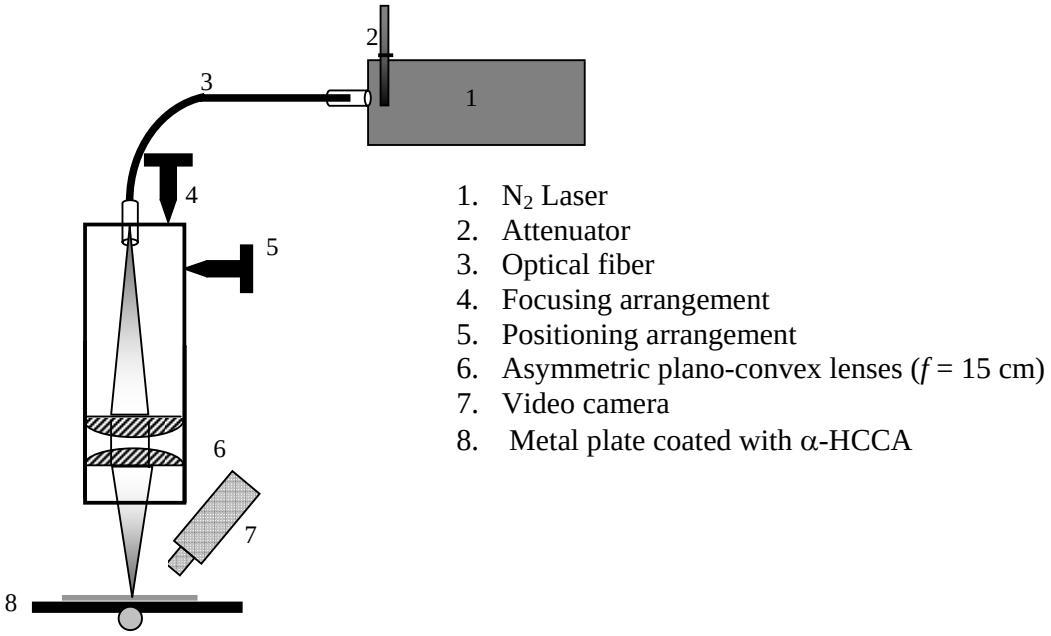

Figure 2: Laser delivery system to focus the spot on to the target

In this experiment, a pulsed N_2 laser (335 nm/ maximum repetition rate 20 Hz) was used as the radiation source coupled to an optical fibre(QMMJ-55-UVVIS, OZ Optics LTD, diameter- 200µm, Numerical aperture- 0.22). In the burning of spots on the sample the laser was fired for 5 s at 10 Hz. The beam intensity attenuator located immediately after the exit of the laser, controlled the laser fluence just above the threshold value. The inbuilt lens system of the laser focuses the light into the optical fibre. The lens system, labelled as 6 in the above figure consists of two asymmetric plano-convex lenses with 15 cm focal lengths which focuses the laser beam onto the thin layer of α-HCCA. This objective lens system theoretically produces one to one image on the sample surface for illuminated output surface of the optical fibre.

The laser focusing height was adjusted using the mechanical system coupled to a micrometer screw gauge (least count = 10μm). The video camera was focused onto the sample surface and the adjustment of the focusing height was made by observing the image on the monitor to focus the beam waist onto the surface. Then the focusing height was changed in 50 μm steps about the beam waist of the image.

The burnt spots on the α-HCCA layer were recorded with the help of a microscope (x 4.5 times magnification) and digital camera (x 5 optical zoom). Photographs of spots were then analyzed by the *Digimizer*^{TM} (trial version) software. This package is capable of analyzing the area of an optical image according to its brightness.

3. RESULTS AND DISCUSSION

The ablated spots on the thin layer of α-HCCA by the focused N_2 laser beam duration through 5 s time period is shown in the following figure.

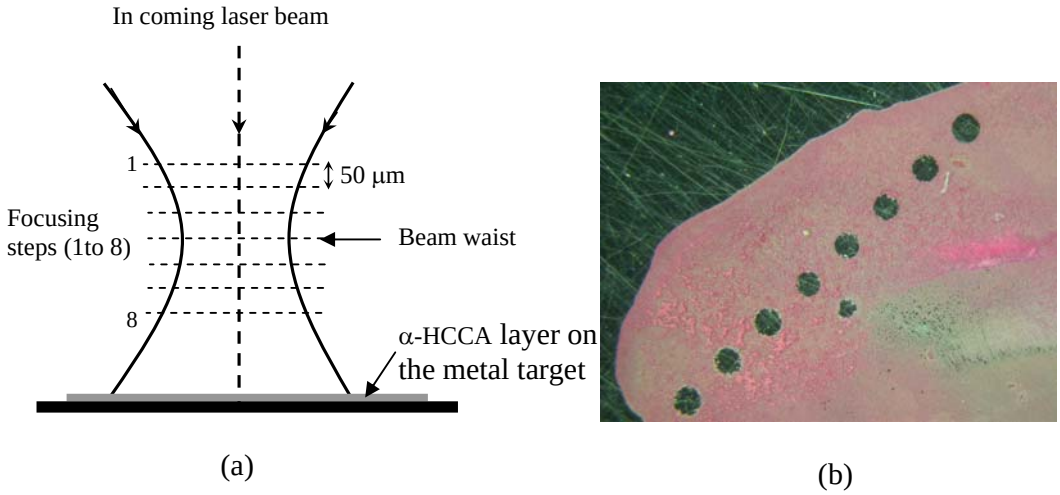

Figure 3: (a) Geometrical diagram to show the focused laser beam, the dashed horizontal lines indicate the 8 number of cross-sections (50 μm steps between each) at which the diameter of laser spots was observed (b) Burnt marks produced by N_2 laser on the thin layer of α- HCCA at the different cross-sections of the beam. The laser fluence was set above threshold

Each spot was recorded on the thin layer of α- HCCA by changing the objective distance in 50 μm steps. In the analyzing of optical image of the spots by the *Digimizer*^{TM} (trial version) first it provides a spectrum of relative brightness of the image. When a desired brightness value is selected on the spectrum, the software marks

the regions in which the brightness is bellow the selected value. The optical image shown in Figure 3(b) was analyzed by the *Digimizer* ™and the result is shown bellow.

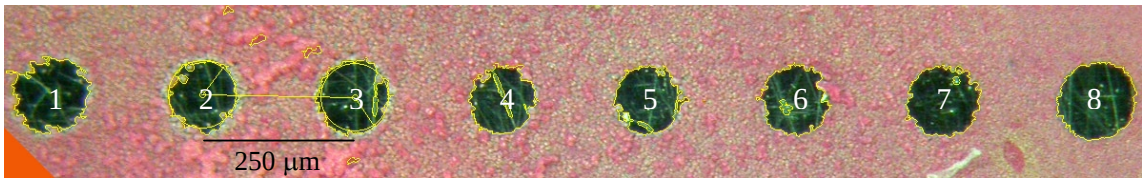

Figure 4: Analyzed burnt marks produced in fig 2 area with *Digimizer*™ (trail version)

The scale of the image was calibrated using the distance between two spots (250 ± 10 µm). The software was advised to determine the area on the image bellow a certain brightness which is corresponding to the brightness of the dark spots. The sharp line at the edge on each spot indicates the boundary of spots determined by the software according to the given requirements. The following table shows the statistics of the laser spots determined by the *Digimizer*™ software marked regions of the image.

Table 1: Statistics of laser spots on the thin layer of α- HCCA determined by the *Digimizer*™ software

Relative height(µm)	Spot area (μm^2)	Calculated Spot diameter(µm)
50	11149	119
100	9635	111
150	10579	116
200	**8743**	**105**
250	**8801**	**106**
300	**9297**	**109**
350	10559	116
400	12616	127

The first column of the above table shows the focusing steps. The spot diameters shown in the third column were calculated according to the spot area determined by the software and considering the shape of each spots as circular. The error in the area of images was neglected because the software is capable of determining the area with the accuracy less than 1 μm^2.

Figure 5 shows the diameter of spots against the focusing height. According to the data, the waist of the focusing beam changes only about 15% in the range of 200 μm of focusing height (relative height; 150 μm to 350 μm). At the fine focusing height, the diameter of the spot is about half of the diameter of the optical fibre (200 μm) which was used to deliver the laser onto the metal target.

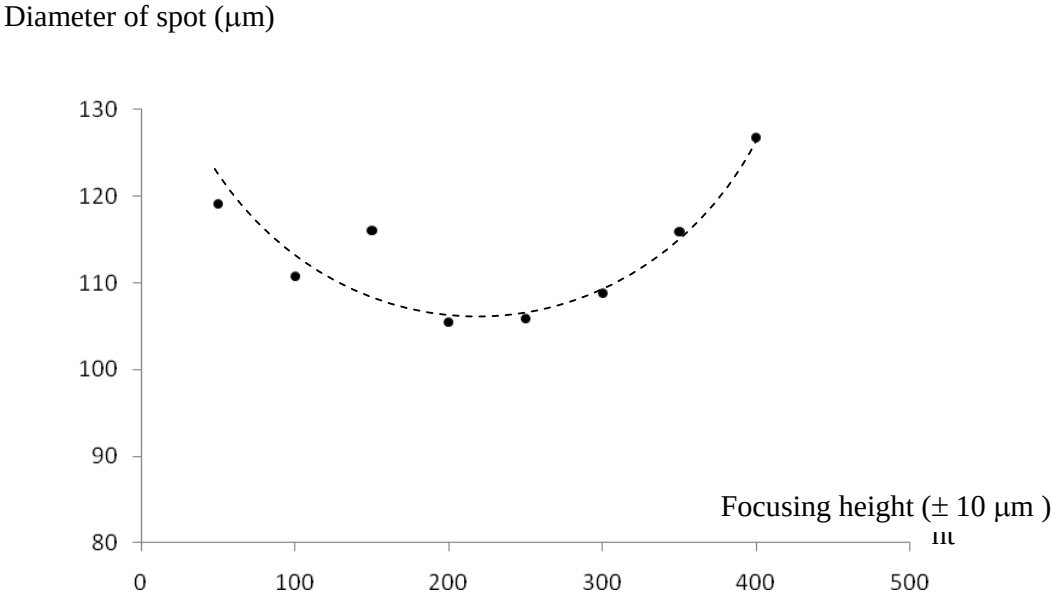

Figure 5: the diameter of spots against the focusing height

4. CONCLUSION

According to the diameter of laser spots on the surface of MALDI target, the diameter of the spot on the target changes about 15 % from its waist diameter (≈105 μm) within the focusing height range of 200 μm distance. If it is assumed that, this phenomena can be generalized for any diameter of optical fibres, a laser spot of 10 μm on the MALDI target in imaging mass spectrometry, changes its diameter to 11.5 μm within 200 μm distance range about its focusing height. This is not a significant change according to the present developed matrix applying techniques in biological tissue imaging mass spectrometry. On the other hand, the focusing of a minimum laser spot on the MALDI target can be achieved with a micrometer scale mechanical tuning arrangement within the above limits of spot diameters.

5. REFERENCES

1. M. Karas, D. Bachmann, U. Bahr, F. Hillenkamp, *Matrix Assisted Ultraviolet- Laser Desorption of Nonvolatile Compounds*. Int. J. Mass Spectrom. Ion Processes, 778 (1987) 53-68.
2. M. Karas, U. Bahr, A. Ingendoh, F. Hillenkamp, *Laser Desorption Ionization Mass-Spectrometry of Protiens of Mass 100,000 to 250,000 Daltan*, Angew. Chem. Int. Ed. Engl.**,** 28(6) (1989) 760-761.
3. A.I. Gusev, O. J. Vasseur, A. Proctor, A.G. Sharkey, D.M. Hercules, *Imaging of Thin Layer Chromotograms Using MALDI Mass Spectrometry*, Anal. Chem., 67(24) (1995) 4565-4570.
4. B. Spengler, M. Hubert, R. Kaufmann, *MALDI Ion Imaging and Biological Ion Imaging with a New Scanning UV- Laser Microprobe*, Proceeding of the 42nd ASMS conference on Mass Spectrometry and ALLied Topics; Chicargo, IL, May, (1994).
5. R.M. Caprioli, T.B. Farmer, J. Gile, Molecular Imaging of Biological Samples: Localization of Peptieds and Protiens using MALDI-TOF MS, Anal. Chem., 69(23) (1997) 4751-4760.
6. M. Stoeckli, P. Chaurand, D.E. Hillahan, R.M. Caprioli. Nat. Med., 7 (2001) 493-496.
7. H. Qiao, V. Fernando, O. Krokhi, V. Spicer, K.G. Standing, and W. Ens, Proc. 55th ASMS Conf. Masss spectrometry and Allied Topics, Indianapolis, USA, (2007)