

MALDI Target Spotting for Proteomics Research

Fast and automated protein characterization is a key issue in proteomics research for drug target discovery. Large numbers of individual proteins are separated by two-dimensional gel chromatography to obtain individual protein spots. Often, the resulting protein spots are then picked, digested and analysed by Matrix-Assisted LASER Desorption-Ionization (MALDI) mass spectrometry. The data obtained from such an experiment include the total protein mass, and — after digestion of the samples with (for example) trypsin — the peptide finger print, a characteristic fragmentation pattern. Combined with the origin of the protein, this information can be compared with the content of databases and is in many cases sufficient for the characterization of the protein under investigation.

To obtain high quality MALDI mass spec data in a fully automated fashion, the proteins have to be spotted on 384-spot metal MALDI targets, such as Anchor Chip plates from Bruker[®]. Whereas in genomics washable steel needles are used for the spotting (e.g., for the quality control of oligonucleotide synthesis), in proteomics, disposable tips are favoured to reduce the risk of cross-contamination and carry-over of the unbound protein samples. Reliable walk-away spotting of flat metal MALDI targets with disposable tips is a challenge for liquid handling systems. It boils down to providing the highest positional accuracy, typically 0.1 mm or better along all axes. Furthermore, 0.5-1 ul of highly organic solvents have to be handled with a high degree of precision.

In the present note, a method is described to obtain protein spots of excellent quality for MALDI mass spectrometry, based on the dried droplet approach.

Experimental Approach

Samples of a test protein are spotted on Anchor Chip MALDI targets from Bruker. Protein spots are analysed under a microscope in terms of position, shape, quantity, and reliability. The samples are spotted using a Hamilton [Microlab STAR](#) pipetting workstation (see figure 1) with CO-RE (Compression-induced O-Ring Expansion) technology. This feature allows for the accurate coupling of needles and disposable tips to the pipetting channel. The cylindrical shape of the coupling section (see figure 2) allows for a

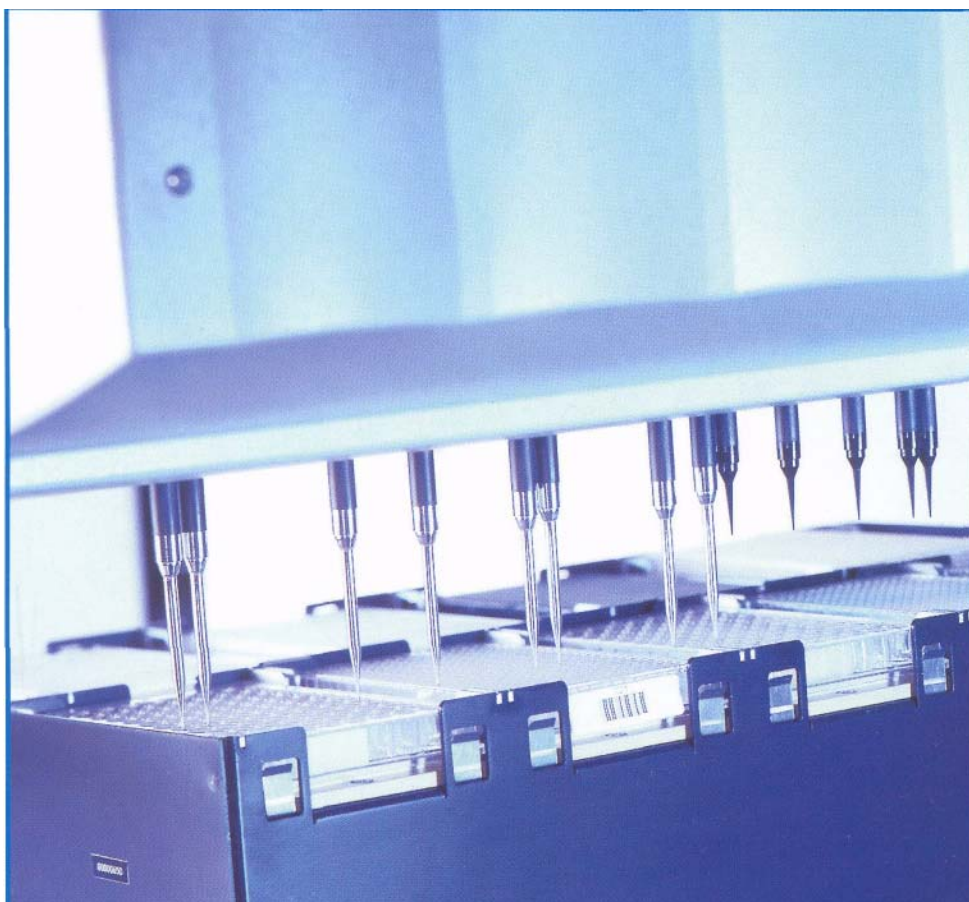


Fig. 1: Hamilton Proteomics STAR workstation.
Caption: Proteomics workstation based on Microlab STAR with CO-RE technology.

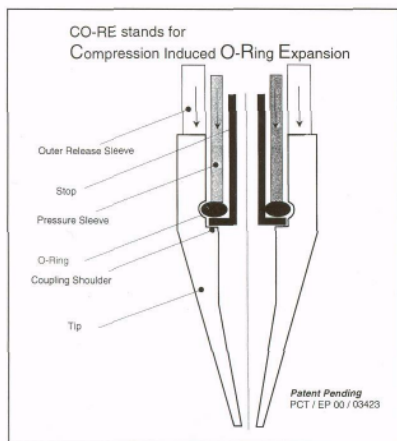


Fig. 2: Hamiltons CO-RE technology.
Caption: CO-RE (Compression-Induced O-Ring Expansion) technology couples pipetting channels with disposable tips or steel needles so as to guarantee the highest positional accuracy (≤ 0.1 mm) along all axes.

positional accuracy of 0.1 mm in x- and /- directions (the target plane). For spotting, the z (touch-off) position is extremely critical. Deviations in the order of 0.3 mm immediately result in a lessening of spot quality. With CO-RE technology, a shoulder within the tip gives precise z-positioning to 0.1 mm. To compensate for the microplate footprint-sized target holder tolerances, the spotting program measures the z positions of the individual spots by capacitance-based liquid level detection and stores the values in an array. Whenever the position is repipetted, the individual z-position is retrieved to guarantee reliable spot quality. Throughout the process, accurate, droplet-free pipetting is achieved by use of 10- μ l disposable filter tips, and aspiration of the organic fluids with pressure-based liquid level detection. Given the many different spotting procedures available, the following approach allow for the most reliable and accurate automation of the process. Figure 3 shows a schematic view of our MALDI target spotting procedure. Typically, 1 μ l of highly organic matrix solution is aspirated, followed by a consecutive aspiration of 0.5 μ l of aqueous protein sample. The individual z-position of the spot is measured (3A), the tip moves up typically by 0.4 mm (3B), and the load is dispensed by touch-off, enabling contact between target, liquid, and tip (3C). Next, a wash step is applied: a fresh tip with an aqueous wash solution dispenses a droplet onto the target (3D). After a variable waiting time, the droplet is re-aspirated from the target (3E), thus de-salting the spot to avoid side bands in the mass spectrum of the protein sample. This circumvents the use of expensive, special chromatographic tips for cleaning. Finally,

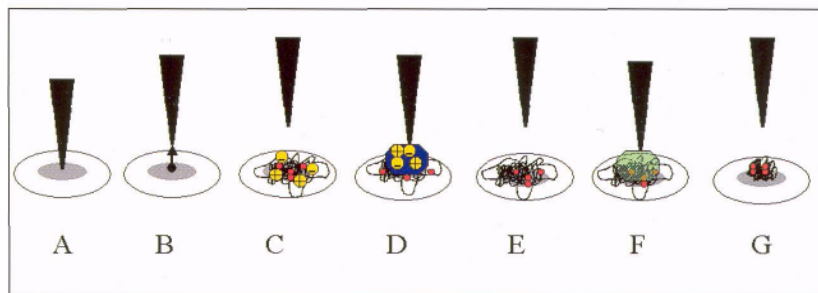


Fig. 3: Protein spotting diagram

Caption: The diagram illustrates the technique of spotting protein samples and a matrix on an Bruker Anchor Chip 384-spot MALDI target.

- A:** The blank spot consisting of a hydrophilic area surrounded by a hydrophobic ring. The precise z-position of the individual spot is measured by capacitance liquid level detection.
- B:** The tip moves up by a defined height, typically 0.4 mm.
- C:** The protein (black coil), matrix (red dots), along with traces of salt (yellow +/-) are spotted on the target.
- D:** Salt is washed away with aqueous solution.
- E:** De-salted protein/matrix mixture.
- F-G:** Dispensing recrystallization liquid on spot to concentrate protein/matrix mixture on anchor.

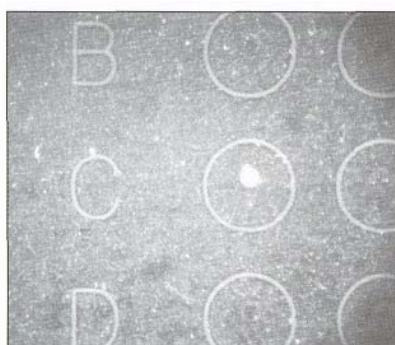


Fig. 4: Protein Spots on Target
A Fig. 4a) shows prepared protein spots in comparison with empty spots.

V Fig. 4b) shows a microscopic view of a single protein and matrix spot, concentrated on the anchor.



a highly organic solvent is dispensed onto the spot (3F), allowing the protein sample to be shrunk to the hydrophilic anchor of the Anchor Chip MALDI target (3G). This increases the sensitivity of the mass spectrometry to the femto (or even sub-femto) molar range³ and allows a fully automated, walk-away analysis. The spots obtained are shown in figure 4. Figure 4a shows two spots before and after dispense for comparison. Figure 4b shows an enlarged view of a typical individual protein spot, perfectly concentrated on the anchor.

The throughput of the 8-channel Micro-lab STAR is roughly one 384-well target every two hours. This could easily be doubled using a 16-channel system, processing two targets at a time.

Conclusions

Hamilton has proposed a method for fully automated MALDI target spotting with maximum reliability and excellent spot quality. Highest positional accuracy with disposable tips, as well as precise surface detection systems, are essential features for guaranteeing excellent spot quality. Further developments are expected to involve automation of a common spot digestion and spotting application on one and the same instrument, thus increasing walk-away times for large scale proteomics target discovery.

Literature

- 1 Automation of MALDI-TOF Analysis for Proteomics, Application Note SMT-50, www.daltonics.bruker.com/applications
- 2 Anchor ChipTechnology for the OmniFLEX ; A Highly Sensitive Bench-Top Instrument, Application Note MT-1070, www.daltonics.brukercom/applications
- 3 Detection Limits of MALDI-TOF PSD for Peptide Sequencing from Protein Digests, Application Note MT-52, www.daltonics.brukercom/applications

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