APPLICATION INFORMATION

ProteomeLab™ Automation

USING THE BIOMEK® FX LABORATORY AUTOMATION WORKSTATION TO INTERFACE THE PROTEOMELAB PF 2D SYSTEM WITH THE MALDI-TOF MS FOR MULTIDIMENSIONAL PROTEOME PROFILING

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Introduction

The discovery stage of proteome profiling typically involves the comparison of different states of a cell or tissue. One approach utilizes fractionation of the proteome into intact proteins, followed by mass spectrometry (MS) analysis. This paper will describe the combined benefits of an advanced two-dimensional, liquid chromatographic fractionation system, the ProteomeLab™ PF 2D, with the ultra-high performance of MALDI-TOF MS analysis for proteomics. The first dimension separation is chromatofocusing where proteins are separated by pI and collected in fractions based on pH intervals. Upwards of 20 pI fractions are then separated in a second dimension by reversed-phase chromatography. From each 2nd-dimension run, 80-90 fractions can be collected. To accommodate the large number of fractions generated by the first two dimensions for subsequent MS analysis, the Biomek® FX Laboratory Automation Workstation was used as the interface between the ProteomeLab PF 2D and MS by spotting the MALDI plate with 2nd dimension fractions. The Biomek FX Laboratory Automation Workstation prepared and spotted the fractions from the 2nd dimension runs along with the appropriate matrix onto a 384-well format MALDI target. The target plate is analyzed directly by a MALDI-TOF MS, which measures the mass-to-charge ratio of the intact proteins as the third dimension.

This paper will illustrate a "proof of concept" by performing automatic two-dimensional separation of human plasma with the ProteomeLab PF 2D, followed by analysis of intact proteins by MALDI-TOF MS as the 3rd dimension (Figure 1).

Materials and Methods

Plasma Sample Preparation

20 mL of blood was drawn from a healthy adult male. The blood sample was collected into tubes containing K_3-EDTA and centrifuged at 1000 rpm for 45 minutes at 8°C. The plasma was carefully removed, aliquoted, and frozen at -80°C. The protein concentration, as determined by biuret assay, was 82.51 mg/mL.

ProteomeLab PF 2D Fractionation

The components, principle of operation, materials, and method of separation of the ProteomeLab PF 2D system have been previously described.[1] The plasma sample was injected at 2.5 mg of protein onto the 1st dimension of the ProteomeLab PF 2D. To equilibrate the plasma sample to the starting pH, 30 µL of plasma were mixed with Start Buffer to a final volume of 400 µL and the entire volume of the sample was injected.

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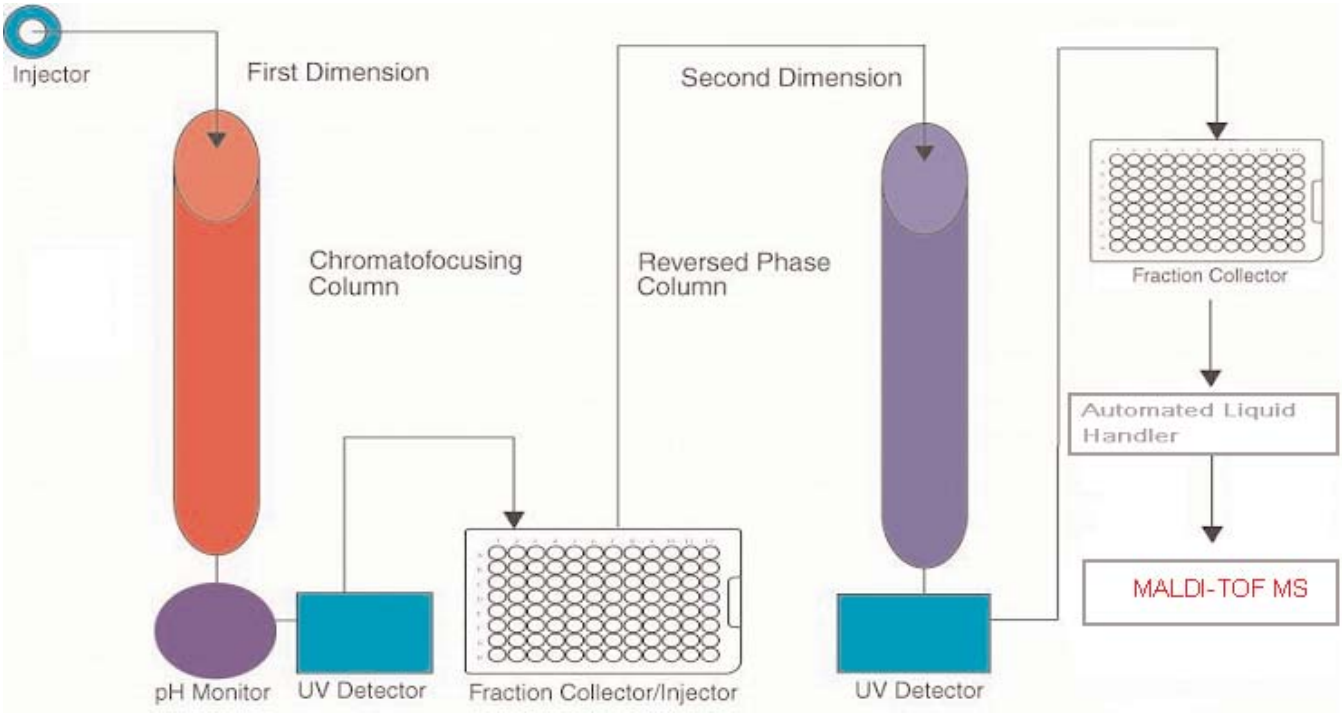


Figure 1. *The workflow for multidimensional proteome profiling with the ProteomeLab PF 2D and MALDI-TOF MS with the Biomek FX as the interface between these.*

2nd-*Dimension Fraction Collection*

Fifteen-second fractions were collected from the 2nd-dimension run of the pH fraction 5.24-5.55. Fractions were collected from 6-24 minutes of the 2nd dimension into a Greiner V-bottom polypropylene plate using the Gilson FC 204 Fraction Collector (see Appendix A for a complete list of part numbers).

Spotting MALDI AnchorChip with the Biomek® FX

Materials

The matrices and the metal target surface used for the MALDI-TOF MS were obtained from Bruker Daltonics. Two matrices were used: α-cyano-4-hydroxy-cinnamic acid (HCCA) and sinapinic acid (SA). The metal target was the AnchorChip* with 384 targets that each has an anchor diameter of 600 µm for spotting sample and matrix. The AnchorChip 600/384 target required an adapter frame for automation purposes.

The Biomek FX version 2.2 (Build 6) was the robotics controlling software for the Biomek FX Laboratory Automation Workstation (Figure 2). A positive-positioning ALP (automated labware positioner) was required on the Biomek FX to hold the AnchorChip target plate in place to maintain accurate pipetting. Other hardware used for the automated method included the Biomek FX 96-Channel Disposable Tip Pipetting Head -Low Volume, Biomek FX Disposable Tip Loader ALP, 2 Standard Single-Position ALPs, and a High-Density Sixteen-Position ALP. Labware used in this method included AP96_20 µL tips and Greiner 96 U-bottom polypropylene plates.

The matrix solutions used the following solvents: acetone, reagent grade; ethanol, reagent grade; acetonitrile, HPLC grade; trifluoroacetic acid (TFA), HPLC grade; and HPLC grade water. The 0.1% TFA in water was the same as that used as solvent A in the 2nd dimension of the ProteomeLab™ PF 2D.

Preparation of Matrix Solutions

The HCCA matrix solution was prepared by dissolving 9 mg of HCCA in 3 mL of acetone, which was then diluted with 6 mL of ethanol to prepare the working solution. To prepare SA, the matrix solution, acetonitrile was mixed with 0.1% TFA in water (9:1) and 10 mL of this mixture was used to dissolve 20 mg of SA which was the working solution. Aliquots of 100 µL of each matrix working solution were then distributed into each well of a 96 U-bottom polypropylene plate. The best results are obtained when the matrix stock solution is prepared immediately before use.

Preparation of Recrystallization Solutions

Two different solutions were used for recrystallization, depending on the matrix selected. For HCCA matrix, the recrystallization solution is prepared by adding ethanol, acetone, and 0.1% TFA in water at a ratio of 6:3:1 to a total volume of 10 mL. The SA recrystallization solution is made by mixing acetonitrile with 0.1% TFA in water (9:1) to a total volume of 10 mL. Aliquots of 100 µL of the appropriate recrystallization solution were then distributed into each well of a 96 U-bottom polypropylene plate. Best results are obtained when the recrystallization solutions are prepared immediately before use.

Spotting Methods

A method was created to spot 96 samples simultaneously onto the AnchorChip plate with one dispensing step. The method also has the capability to spot the complete 384-well AnchorChip. Without taking into account the drying time, this method took less than 10 minutes to run. There were two parts to the method. The first part was the matrix/sample dispense (Appendix B), which used a Biomek® FX 20-µL tip to aspirate 1 µL of matrix followed by 1 µL of 2nd-dimension fraction and deposit them onto the target together. Depending on laboratory conditions, the matrix/sample spot took approximately 10 minutes to dry completely.

The second part of the method was sample recrystallization (Appendix C). It was required to help concentrate the sample plus matrix onto the center of the anchors. For recrystallization, 1 µL of the appropriate recrystallization solution was dispensed onto the dried spot to redissolve the crystals and center the sample onto the anchor. The plate was then allowed to dry for an additional 10 minutes. Once the AnchorChip plate was dried completely, it was then ready for MS analysis.

MALDI-TOF MS Analysis

The m/z values were determined for the intact proteins from spotted 2nd-dimension fractions with a Reflex III* MALDI-TOF MS.

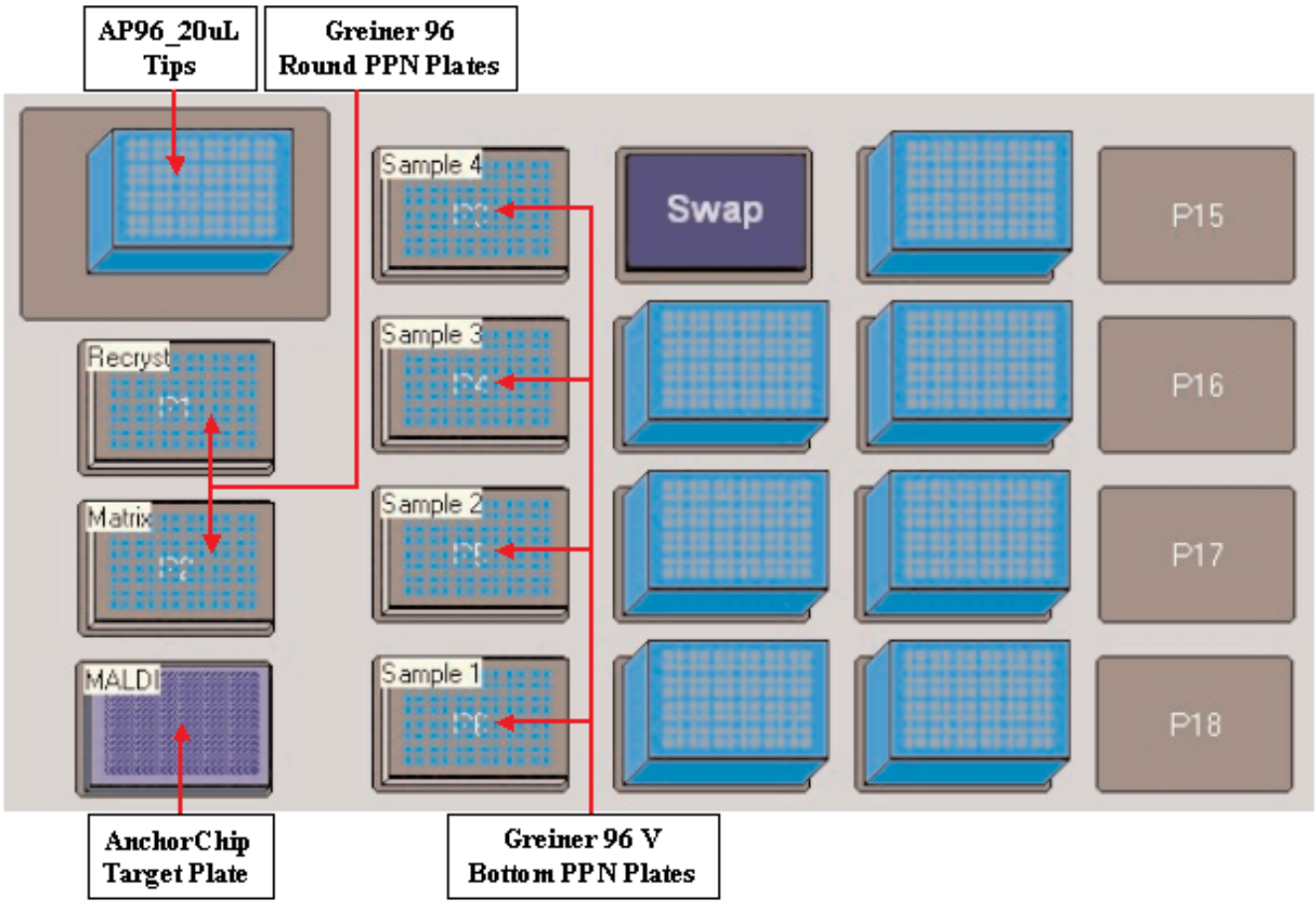


Figure 2. *Instrument setup of the Biomek FX for the AnchorChip Target MALDI spotting method.*

Results and Discussion

The ProteomeLab™ PF 2D effectively fractionates proteomes for subsequent MS analysis. Since the fractions are liquid, no sample solubilization is required prior to MS. One of the 1st dimension fractions from human plasma, which has a pH range of 5.24-5.55, was run on the 2nd dimension (Figure 3). Fifteen-second fractions were collected from the 2nd dimension run and an aliquot of each fraction was spotted with the Biomek® FX onto the MALDI target plate for analysis by MS.

With the HCCA and SA matrices, a prominent peak was observed in the MS spectra from the intact proteins of this 2nd dimension run. The peak, which had a 2nd dimension retention time of 19.00-19.25 minutes, had m/z values for HCCA and SA of 28,567 and 28,177, respectively (Figure 4, 5). This retention time corresponds to that for human apolipoprotein A1 on the 2nd dimension and the m/z values correspond to that of the single charged species of this protein. Subsequent MALDI-TOF MS analysis of tryptic fragments of this fraction identified this protein as human apolipoprotein A1 (data not shown). The theoretical pI value of secreted human serum albumin is 5.27,[2] which is within the pH limits of the starting 1st dimension fraction. Human apolipoprotein A1, is the major protein component of high density lipoprotein. Given its role in binding lipids, it is not surprising that apolipoprotein A1 has a retention time that is three minutes greater than serum albumin, although the former is less than one half the molecular weight. This demonstrates the selectivity of the ProteomeLab PF 2D 2nd-dimension separation based on percent hydrophobicity.

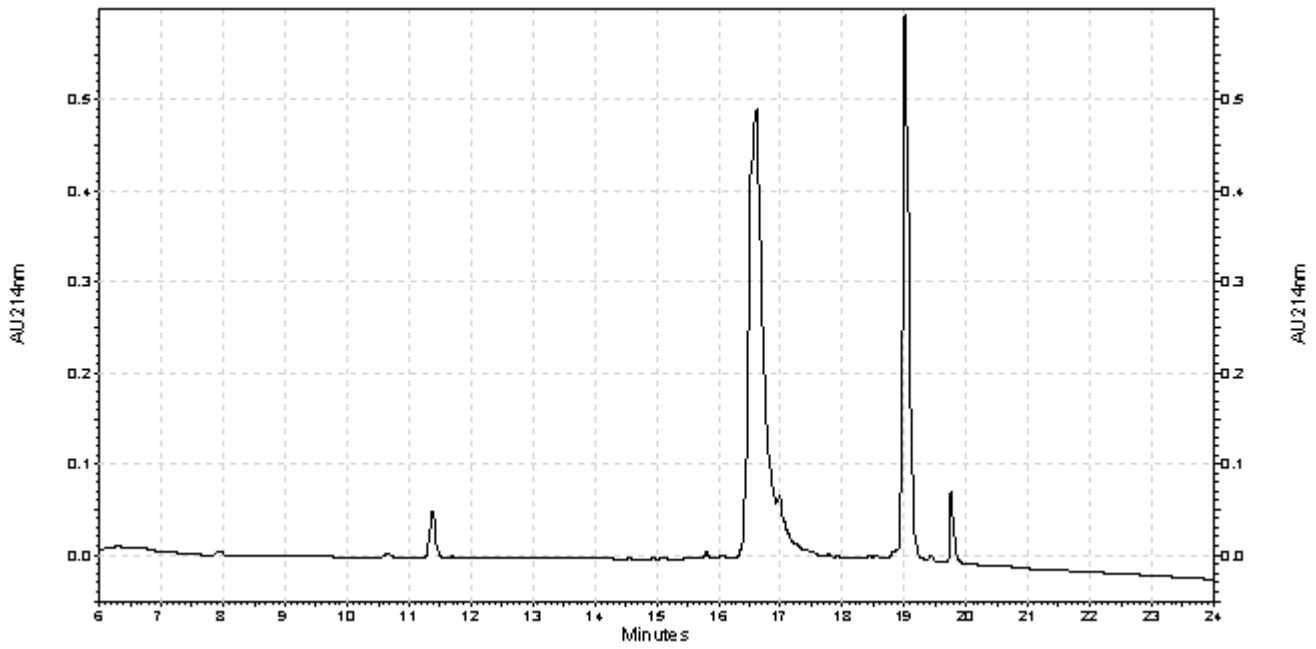


Figure 3. *The 2nd-dimension chromatogram of pH fraction 5.24-5.55 from human plasma sample. Fifteen second fractions were collected between 6-24 minutes for MALDI-TOF MS of the intact proteins. The intact protein peak at 19.1 minute was analyzed by MALDI-TOF MS.*

Summary

The combination of ProteomeLab™ PF2D separation of complex protein mixtures and MALDI-TOF MS is an advanced analytical tool for proteomics. MALDI-TOF MS analysis of 2nd-dimension fractions gives information on exact masses of intact proteins. To interface this combination, the Biomek® FX Laboratory Automation Workstation was used. Even with the volatile working matrix and recrystallization solutions, the pipetting of 1 µL volumes these solutions was very reproducible with the Biomek FX Laboratory Automation Workstation. The results obtained by MALDI-TOF MS show the m/z values corresponding to the mass of human apolipoprotein A1 with both matrices. The Biomek FX Laboratory Automation Workstation facilitated the complete analysis of a fractionated proteome by removing the potential bottleneck resulting from the large number of samples collected from the first two dimensions.

References

1. Simonian, M. H, Betgovargez, E. "Proteome Analysis of Human Plasma with the ProteomeLab PF 2D System." Beckman Coulter, Inc. *Application Information Bulletin A-1963A* (2003).
2. SWISS-2DPAGE database, *http://www.expasy.ch/ch2d/*

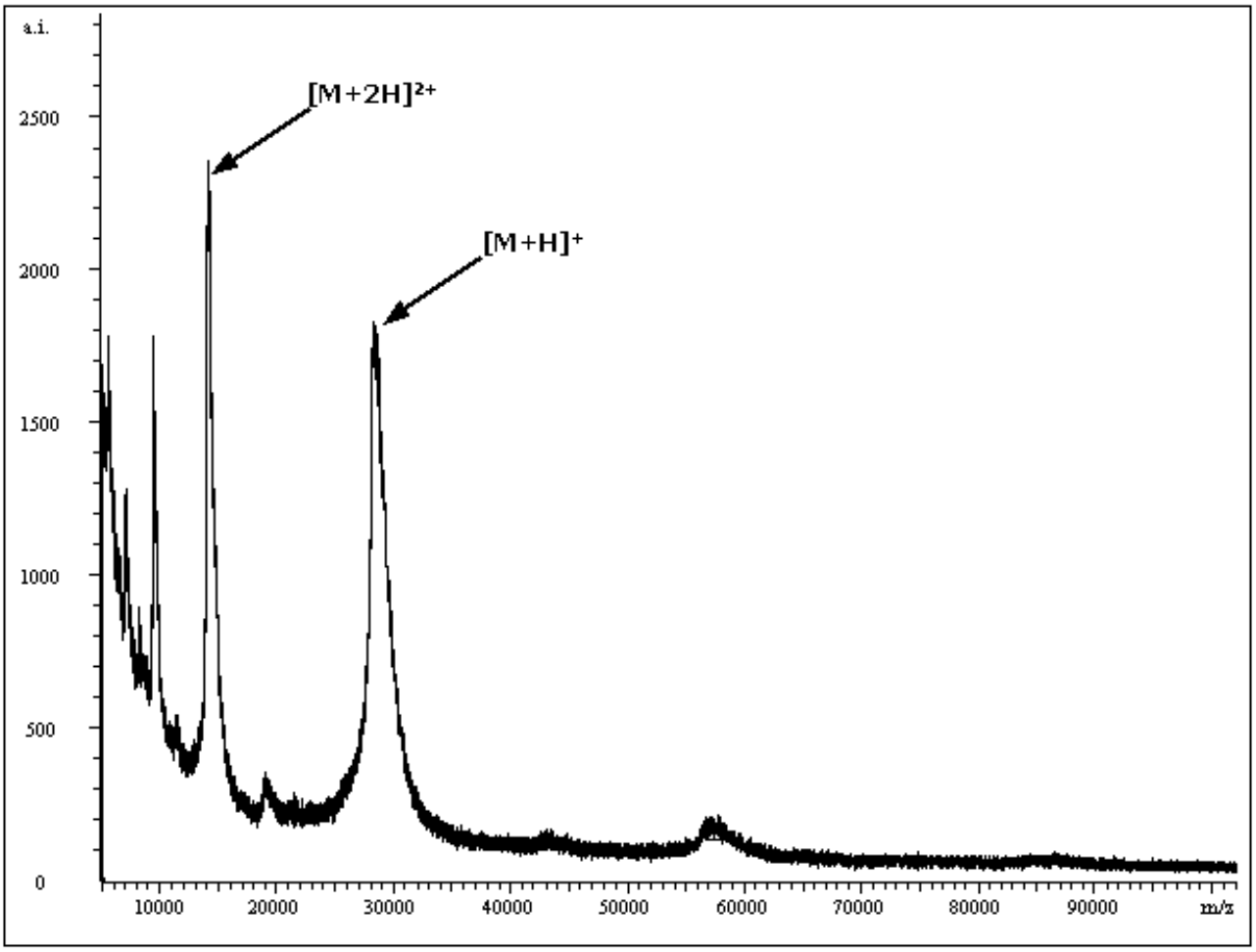


Figure 4. *This MALDI-TOF MS spectrum of the 2nd-dimension fraction that corresponds to the retention time of 19.00-19.25 min in Figure 3. The single charge species has a m/z value of 28,567. The fraction was spotted using HCCA matrix.*

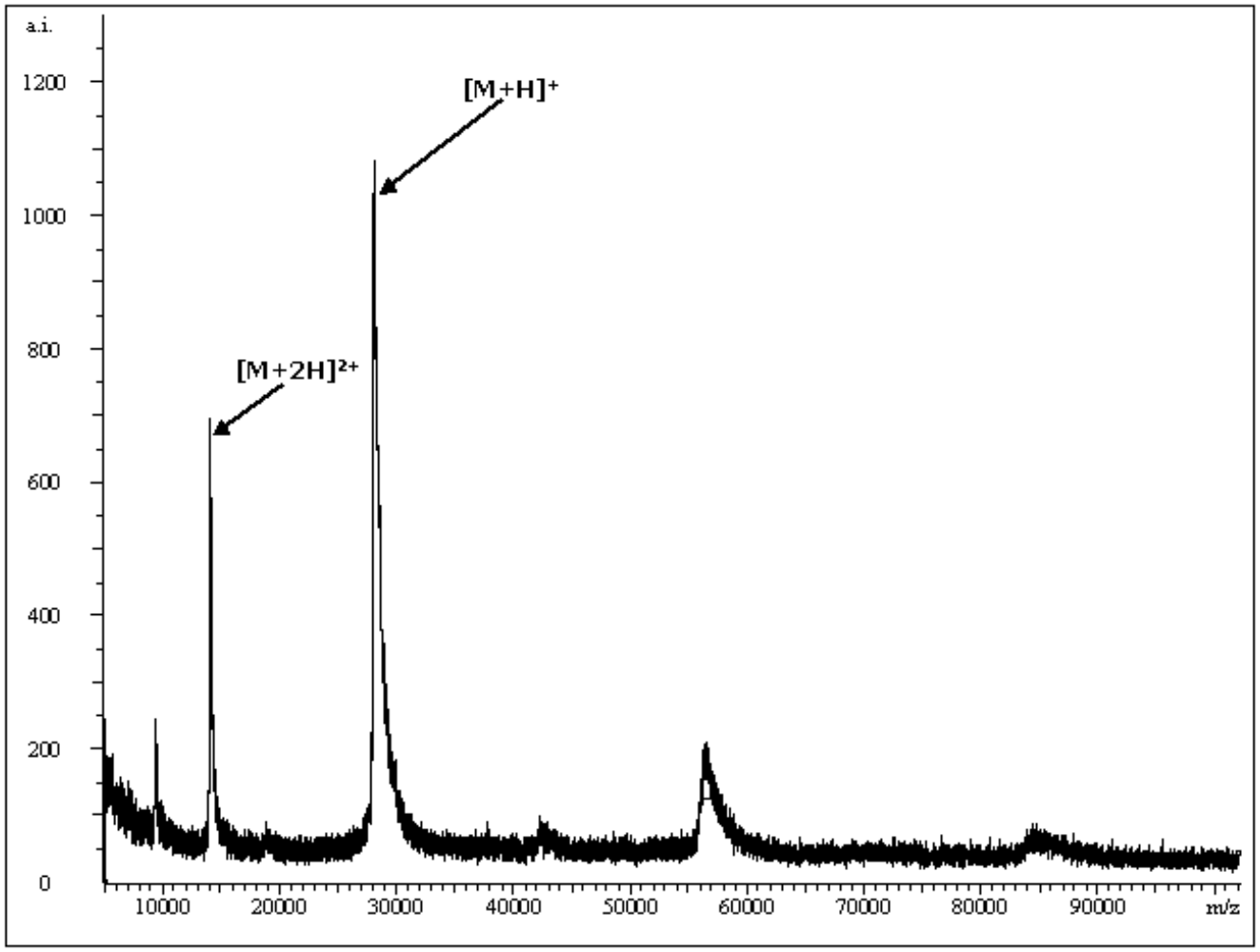
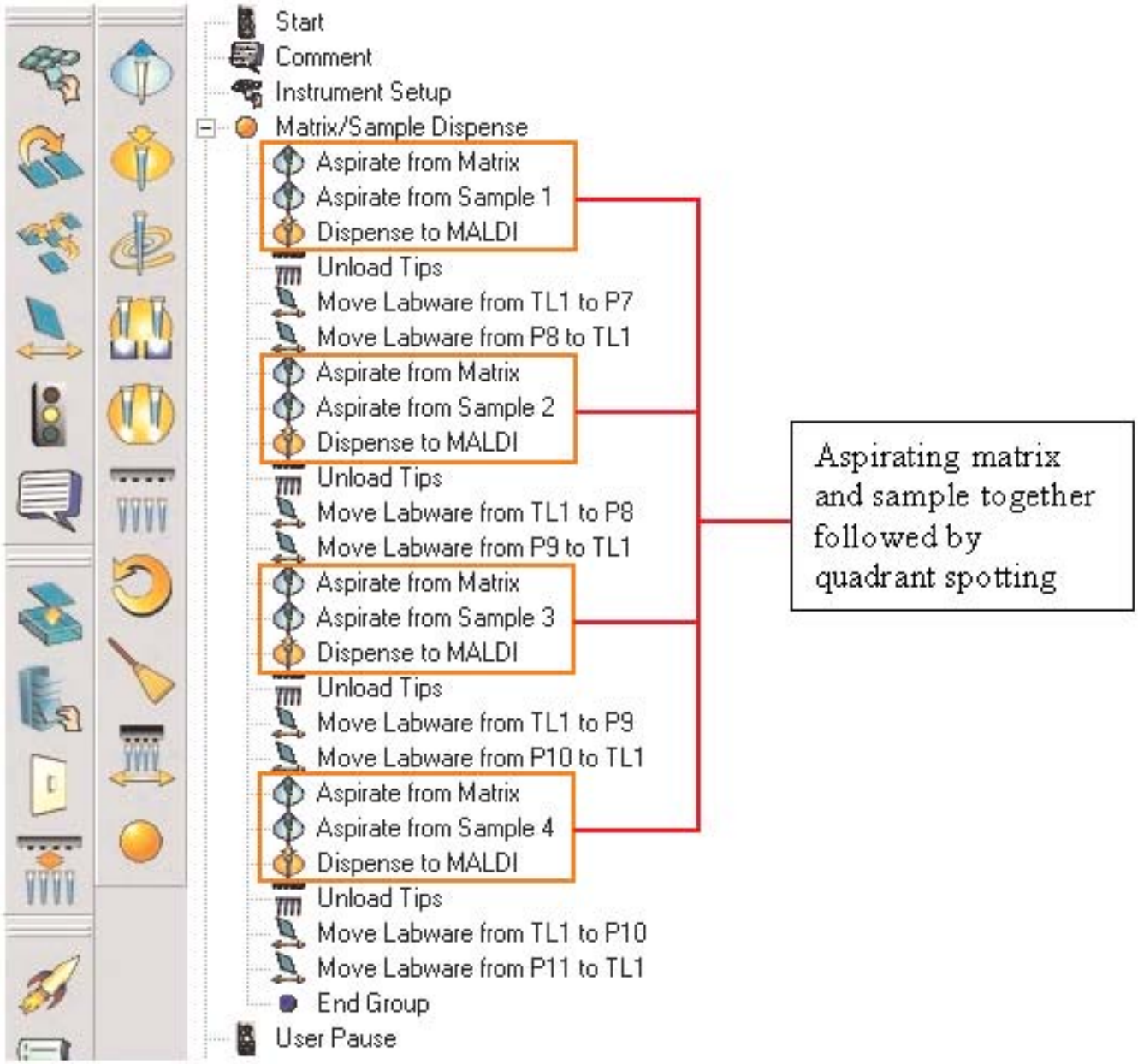


Figure 5. *This MALDI-TOF MS spectrum of the 2nd-dimension fraction that corresponds to the retention time of 19.00-19.25 min in Figure 3. The single charge species has a m/z value of 28,177. The fraction was spotted using SA matrix.*

Appendix A: List of Part Numbers

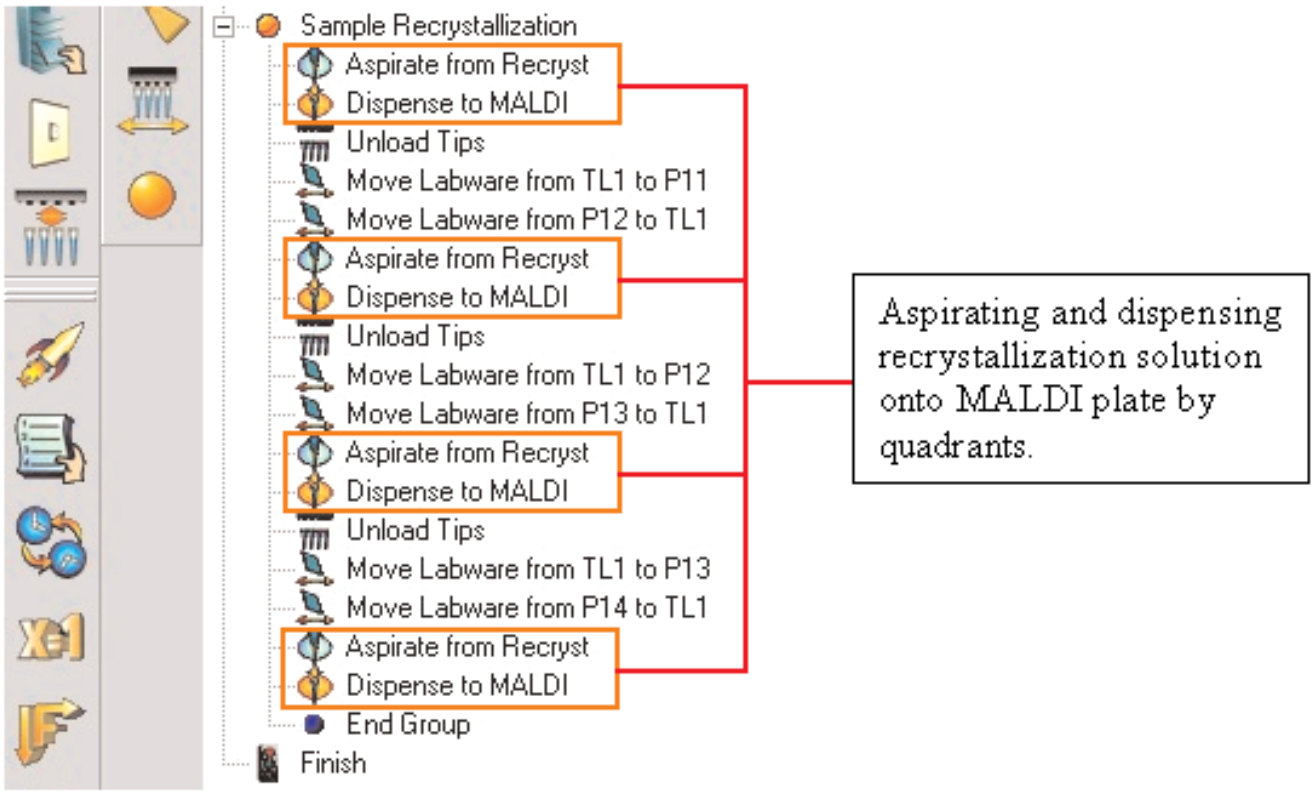
Biomek FX Materials		
Part Number	***Description***	
719349	Biomek FX Software	
719856	Positive Positioning ALP	
719367	Biomek FX 96-Channel Disposable Tip Pipetting Head-Low Volume	
719356	Biomek FX Disposable Tip Loader ALP	
719357	Standard Single-Position ALP	
719360	High-Density Sixteen-Position ALP	
717255	Biomek AP96 P20 Tips, Sterile	
Materials Not Provided By Beckman Coulter, Inc.		
Part Number	***Description***	***Vendor***
201344 (1g) 203072 (5g)	-Cyano-4-hydroxy-cinnamic acid (HCCA)	Bruker Daltonics
201345 (1g) 203073 (5g)	Sinapinic acid (SA)	Bruker Daltonics
73021	AnchorChip 600/384	Bruker Daltonics
74115 or 204741	Adapter frame	Bruker Daltonics
650201	96-well U bottom polypropylene plate	Greiner bio-one
651201	96-well V bottom polypropylene plate	Greiner bio-one

Appendix B: Spotting Method on the Biomek FX (Part 1)



NOTE: *The first part of this automated method is created to spot up to four sample plates. However, if the total amount of sample plates is less than four, then revise the method accordingly. For example, if two sample plates are used for spotting AnchorChip MALDI plate, then delete those steps required to spot sample plates 3 and 4 as highlighted in the above figure.*

Appendix C: Spotting Method on the Biomek FX (Part 2)



NOTE: *The second part of this automated method is created to perform recrystallization of up to four quadrants from the AnchorChip target plate. However, if the total amount of sample plates is less than four as determined in part 1 of the method, then revise it accordingly. Using the same example in Appendix C, if the steps created to spot sample plates 3 and 4 are deleted, then recrystallization of quadrants 3 and 4 are not needed also. In other words, only do recrystallization to those quadrants spotted.*

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