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Digitized Nanobore LC-MS Control System with Integrated Emitter Divert & Rinse for Improved Nanospray Performance

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Introduction

Nanospray is an essential tool in high-sensitivity mass spectrometry (MS), however limited robustness and reproducibility challenge quantitative application. Current trends toward MS-based biomarker quantitation apply strict requirements on analytical performance of nanobore liquid chromatography (LC)-MS. A vision-based, self-tuning source for use with gradient chromatography was developed to automatically control the electrospray source (Valaskovic, et al. JASMS, 2004, 15, 1201). Here we report the extension of this method with an automated ESI emitter divert-and-rinse functionality. Vision-based control maintains the spray in an optimized inlet position. A digital trigger control switches the emitter between data acquisition and diverted rinse positions. The impact of this system on figures of merit related to qualitative and quantitative nanobore LC-MS are investigated and applied to a pulmonary biomarker study.

Methods & Materials

Mobile phase was delivered by gradient nanobore LC (UltiMate™, Dionex) A linear ion trap mass spectrometer (LTQ™ or LTQ-FT™, Thermo Electron) was fitted with a custom nanospray source (PV-550, New Objective). A USB CCD camera video microscope, positioned orthogonally to the emitter, fed images into a PC. Tip-to-inlet position control was provided by a stepper motor XYZ stage on the source (Digital PicoView®, New Objective). An image acquisition and positioning program (Developed with LabView™, National Instruments) provided control. The emitter position was under full control of the PC. The camera's digital input controlled the emitter position. Contact closure from the MS (or LC) moves the emitter from the acquisition to divert position. The divert position was configured with an emitter rinsing station fed by the MS instrument's syringe pump providing a rinse of the emitter's exterior surface in between sample injections. Two additional software modules were developed to increase user control of the nanospray source and sample injection scheme. A stage method module provided a spreadsheet-style user interface to control the precise (to 10 µm) X, Y, Z position of the emitter at defined time points within the method. A valve control module that also used a spreadsheet-style interface, provided control of up to three nano-injection valves (Upchurch® Scientific) throughout the run duration.

To examine Digital Picoview robustness in a shotgun proteomics workflow, 400 µg of protein extracted from pulmonary artery (provided by Dr. Jerry Eu of Duke University Medical Center) was analyzed using a 2-dimensional peptide separation approach. This pulmonary artery protein extract was digested with trypsin. Tryptic peptides were analyzed via a 2-Dimension separation (IPG-IEF/LC-MS/MS). Isoelectric focusing of peptides was performed using a 24 cm pH 3.5 to 4.5 IPG strip. The

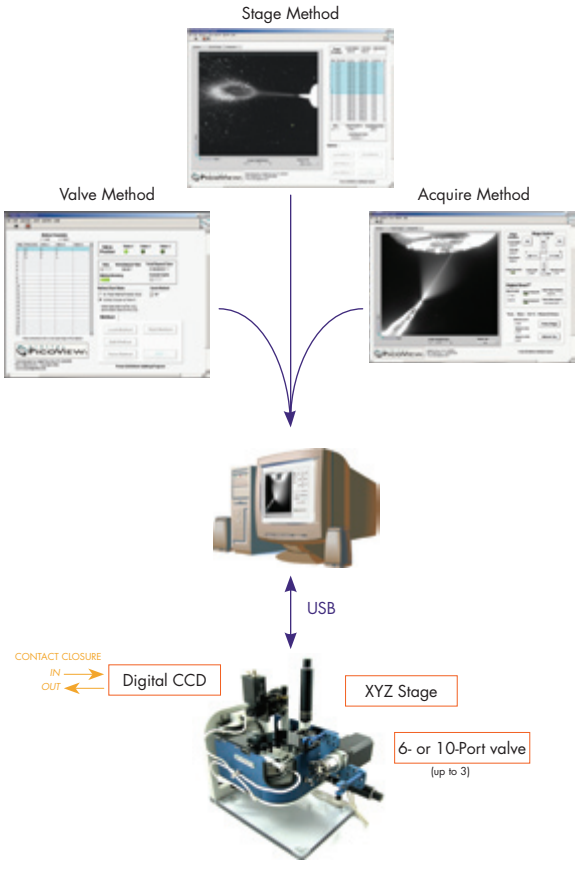


Figure 1 *Schematic of the Digital PicoView®*

second dimension separation was online reverse phase nano-LC-MS/MS (using in house packed trap columns and a 10 cm New Objective PicoFrit® integrated column/ tip packed with ProteoPep™ II C18 media. The nano-LC-MS/MS system was attached to the LTQ linear ion trap mass spectrometer. The resultant data searched against the human International Protein Index (IPI) protein database using SEQUEST™ (Thermo Electron). SEQUEST results were analyzed using custom in-house software for reverse database and orthogonal pI filtering (ID Sieve).

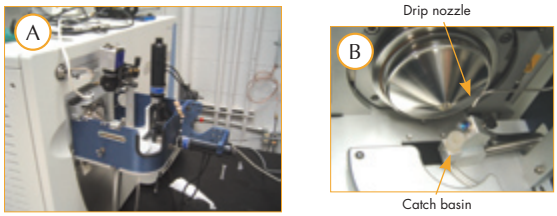


Figure 2 *A) Source mounted on LTQ™. B) Detail of emitter rinsing station.*

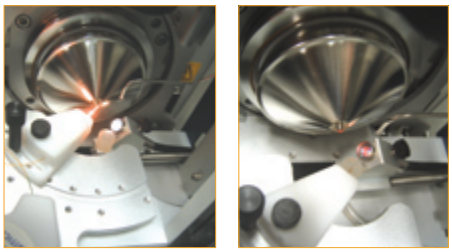


Figure 3 *Digital Divert™ program provides automated emitter rinsing between sample runs. Both the emitter and LTQ™ inlet remain clean by eliminating build-up of salts, etc.*

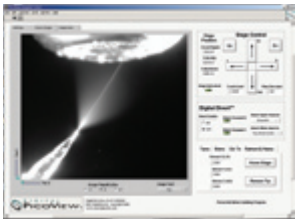
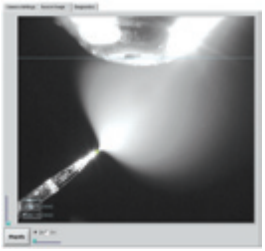


Figure 4 *Source tuning and acquisition module. The emitter position is under full user control via software. The image acquired by the USB camera provides digital magnification to simplify voltage tuning. The Digital Divert™ mode provides for automated tip retraction on a contact closure event.*

Figure 5 *Digital image acquisition window. Calibrated cursors enable precise, repeatable positioning of the emitter relative to the inlet. The distance between any two image points is indicated in mm. Digital image magnification provides facile tip positioning and spray tuning.*



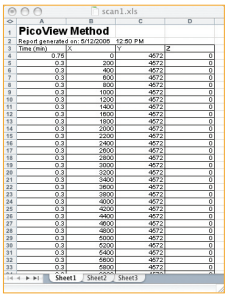


Figure 6 *Method Module™. The emitter position is fully automated via a timed method. X, Y, Z and time values define the emitter position during a run. Methods are readily created in a Miscrosoft® Excel™ Worksheet and imported into the method module. The Method Module can either generate a contact-closure run-through or wait for a contact-closure control via another instrument.*

Figure 7 *Valve Method™ Module. Valve state and time values are used to actuate valves during a run. The valve module can either generate a of a valve switch or wait for a contact closure for control by another instrument. Here is shown the use of the valve module to control two valves for trapped sample injection using a valve mounted on the source, rather than the valve on the autosampler to reduce system volume.*

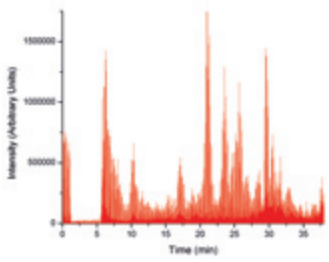


Figure 8 *Representative chromatogram from a multi-dimensional separation of a pulmonary arterial protein shotgun digest. A total of 23 nLC-MS/MS runs from different fractions isolated on sections of IPG strips resulted in identifying more than 1,200 proteins.*

Observations & Conclusions

Control software enables a cursor-based user interface for measurement of emitter and spray plume positions relative to the position of the spectrometer inlet to establish a relative coordinate system. The imaging system measured this reference plume position and enabled repeatable positioning of spray plumes for extended acquisition periods (days) to a spatial volume > 50 μm^3. Tip length variations (of up to 5 mm) with different emitters were normalized to the same spray position. The XYZ stage automatically moved between the (reference) spray position and the diverted (rinse) position. Control of emitter position (spray vs. divert) was determined through reading the value of a digital input available through the camera's interface. An LC-MS-directed command (via contact closure) was then able to keep the emitter in the spray position during signal acquisition periods only. The emitter was maintained in the diverted position during column equilibration. Organic mobile phase (80% MeOH, 1% formic acid), applied to the emitter tip via hanging droplets from conventional 1/16" diameter tubing, was effective at reducing the amount of residue build-up on the outer surface of fused-silica emitters. Elimination of build-up was found to enhance spray stability and minimize variation in applied ESI voltage required for spray stability. Results for average ion intensity and injection peak area (in a non-chromatographic flow injection mode) showed standard deviations of 5% or better for intra-tip performance and 10% or better for inter-tip performance on peptide standards using manual (0.8 µL) injection. The impact of the divert-and-rinse mode on long-term robustness of the nanospray emitter is under further investigation.

In an analysis of the first 23 fractions from the IPG strip 3,085 peptides representing 1,285 proteins were identified to meet filtering criteria. This large number of high-confidence peptide identifications demonstrates the dynamic range and throughput of this separation approach.

Acknowledgement
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