



# Breaking the NanoLC-MS Throughput Barrier: High-Performance at 95% Duty-Cycle

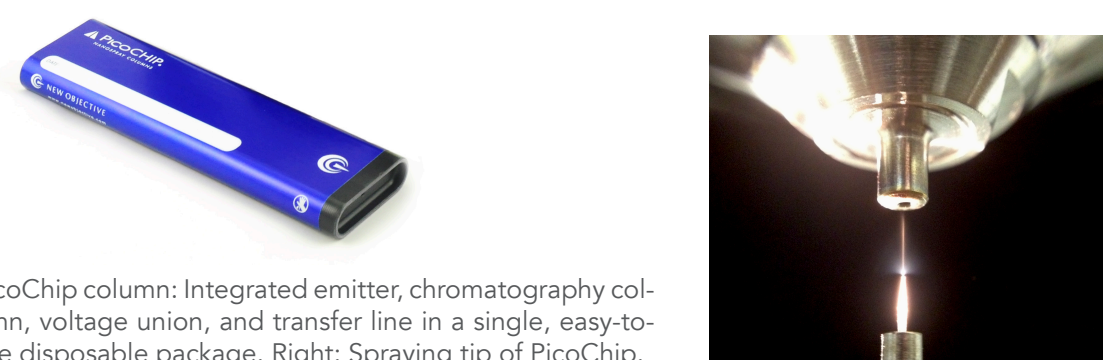
New Objective, Inc.

Booth 153

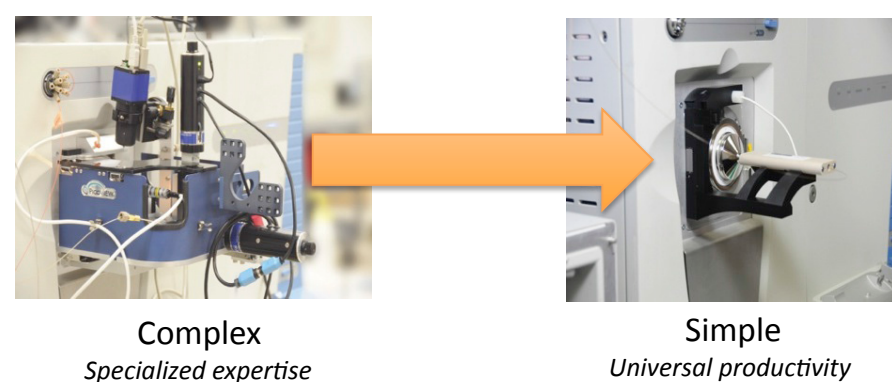
## Introduction

Nanospray is an essential tool in high-sensitivity mass spectrometry, but limited robustness, reproducibility, and ease-of-use have historically challenged the adoption of nanospray in quantitative applications. Recent trends toward MS-based biomarker quantitation have placed strict requirements on the analytical performance of nanobore LC-MS. Nanospray MS and nanobore LC-MS both rely heavily on nanospray source hardware for successful experiments. Nanospray source hardware has matured over the past ten years from simple homemade devices to sophisticated, application-specific instrumentation featuring stage automation, thermal control, and high-resolution imaging. Many of these enhanced features provide robustness (automated tip rinsing, automated emitter change), throughput (multi-channel workflows), ease of use (multi-chip systems) or experimental flexibility. Here we present a novel nanospray source solution which delivers enhanced features of stage automation, multi-channel operation and thermal control. Ease of use has been realized through the incorporation of an integrated nanobore LC-MS consumable (PicoChip®), on which over 400 replicate injections were collected with no loss of chromatographic performance. A four channel, three column version of the source (The PicoSlide™) enables an MS-duty cycle-time of 95%, compared to 40% for a single channel system.

## PicoChip: Easy-to-Use High-Performance Nanospray

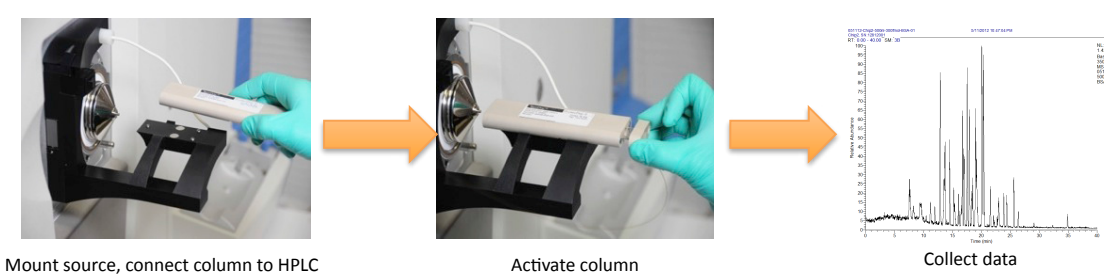


PicoChip column: Integrated emitter, chromatography column, voltage union, and transfer line in a single, easy-to-use disposable package. Right: Spraying tip of PicoChip.



Complex  
Specialized expertise

Simple  
Universal productivity



Mount source, connect column to HPLC

Activate column

Collect data

## Why Attempt Multi-channel LC for Protein or Peptide Analysis by nanoLC-MS?

LC-MS cycle time is long, typically > 15 minutes

- Injection times are long  
Injection volume ≥ column volume
- Gradient delay can be significant: 5-10 minutes  
System flow-rate-to-volume ratio is poor
- Strict requirement on LC peak capacity  
Complex samples, significant mass interference

## Experimental Setup

Liquid Chromatograph: Eksigent Ultra Nano LC – 3 Channel

- Channel 1: loading pump, 1 µL/min, 2% Acetonitrile
- Channel 2: gradient elution pump, 300 nL/min, 2-30% acetonitrile
- Channel 3: washing pump, 1 µL/min, 80% acetonitrile

Autosampler: CTC Leap

- Nano-rotor injection valve, 1 µL inj. volume

ESI Source: New Objective PicoSlide source with three columns.

- PicoChips: 75 µm ID x 10 cm, C18 (Reprosil 3 µm)
- Custom Valco nano-switching valve
- Stage and valve acontrolled by custom Digital PicoView software on host PC

Mass Spectrometer: Thermo Scientific LTQ

- Operated in full-scan mode (300-1200 m/z)
- ESI voltage: 2.1 kV, 180° C inlet temperature

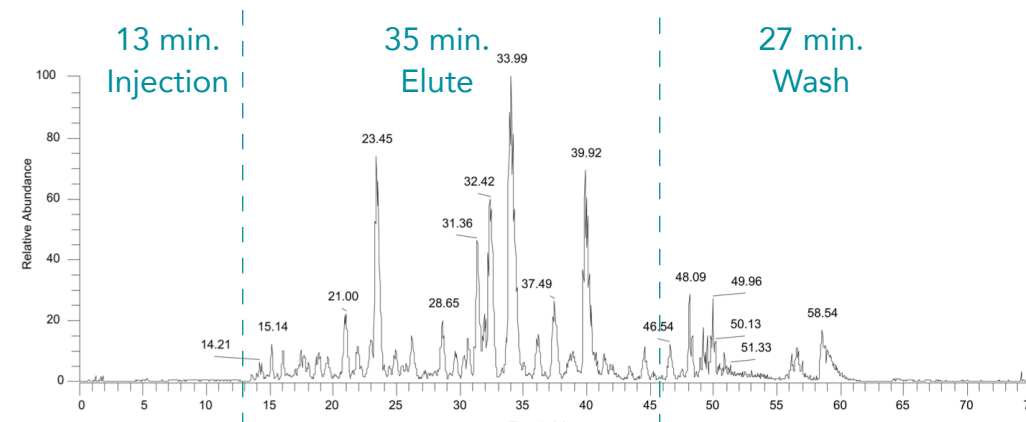
## Conclusions

- Multiplexed operation improves MS duty cycle to be greater than 95%.
- Highly efficient, long injection times are fully supported, eliminating the need for inefficient trapping columns.
- The PicoChip format enables easy multiplexed operation and rapid column switching.
- A novel valve rotor design simplifies the plumbing and provides a linear flow path between the pump and column.

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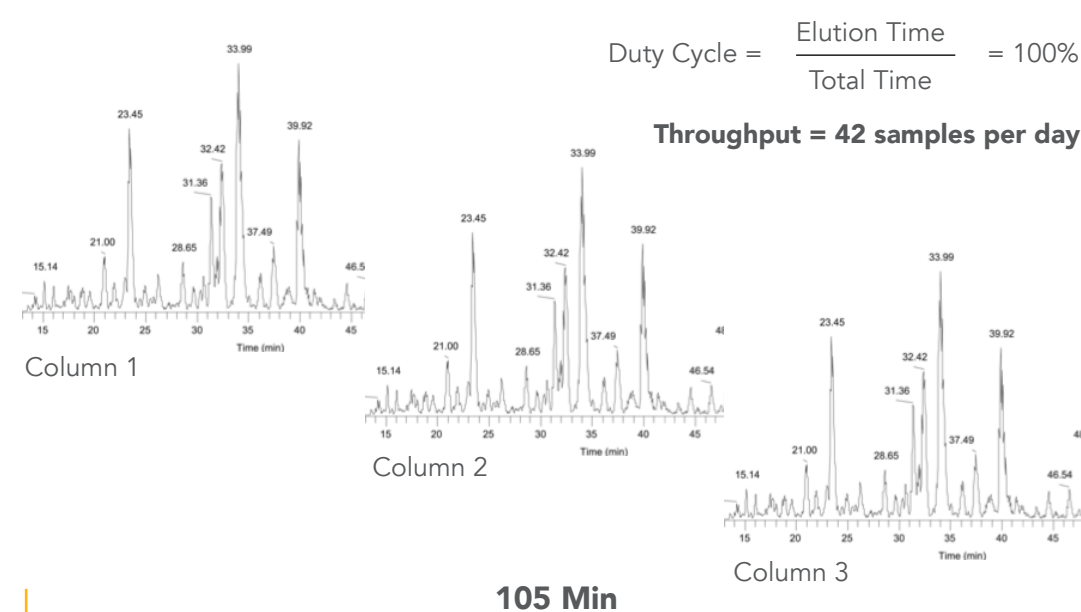
## The Rule of Three

Gradient Elution Has Three Zones

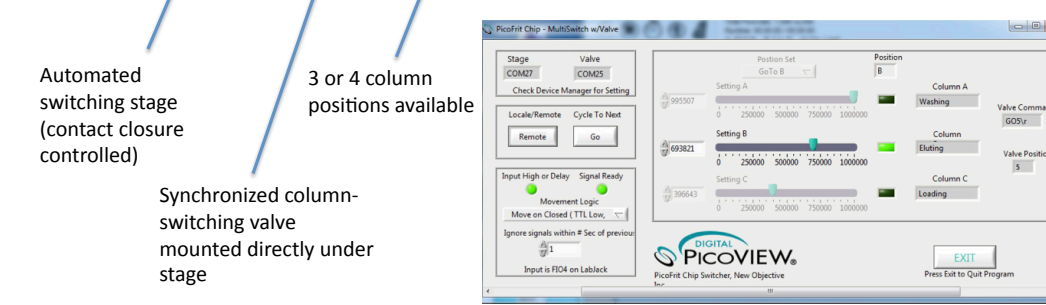


Gradient elution LC has three zones: Injection, elution and wash/equilibrate. MS data is only collected during the elution phase.

Maximized Duty Cycle: Eliminate Injection and Wash Dead Time

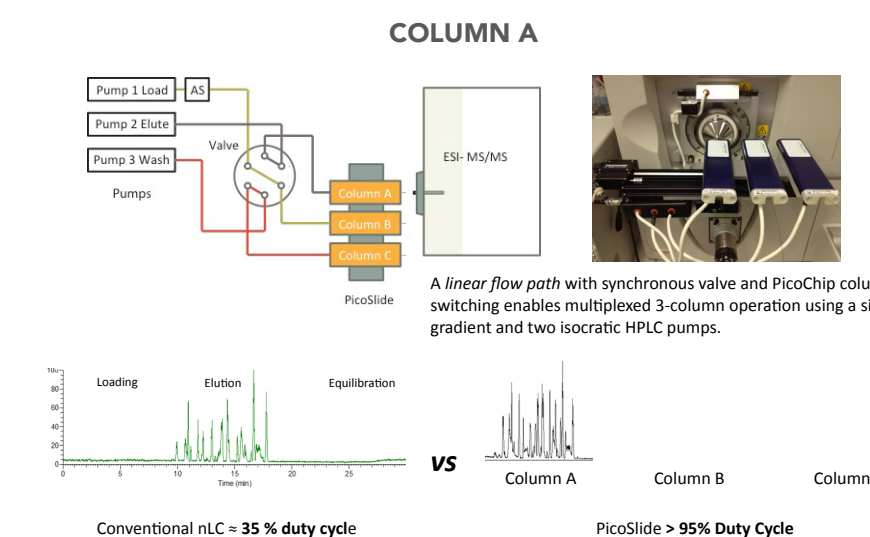


PicoSlide: Advanced Column Switching



Integrated control software for stage and valve

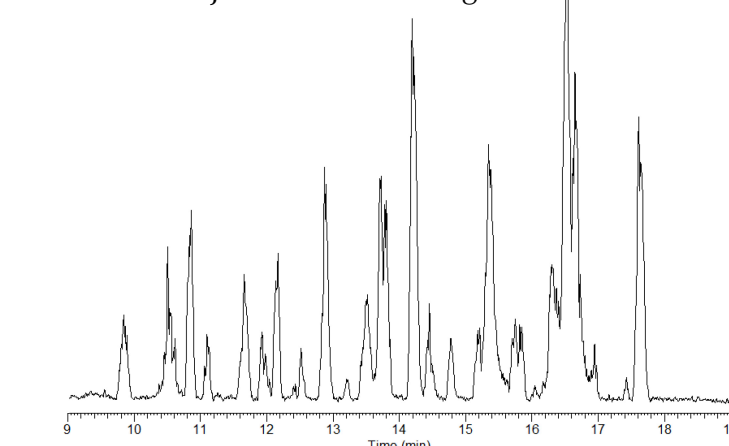
## PicoSlide: Column Switching Results



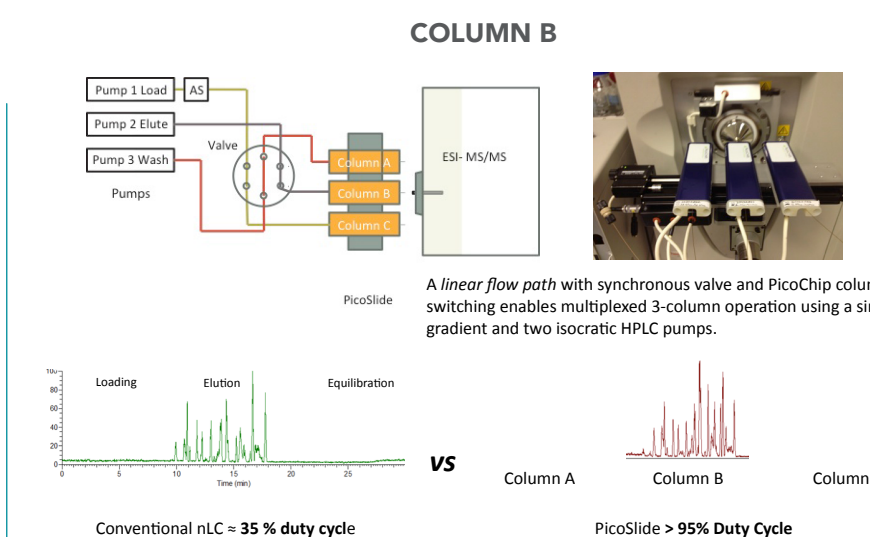
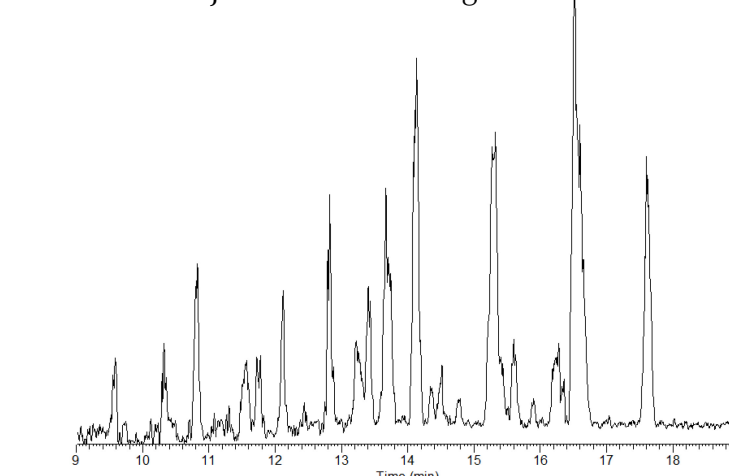
Conventional nLC = 35 % duty cycle VS PicoSlide > 95% Duty Cycle

Collect data from each column only during elution. While Column A is eluting analyte, Column B is loading sample, and Column C is equilibrating for the next injection cycle.

NL: 4.67E7  
INJECTION #5  
300 fmol Injection - BSA Digest



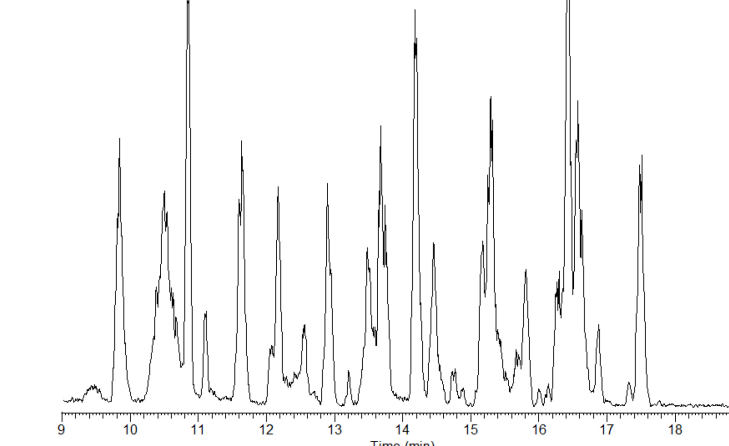
NL: 3.28E7  
INJECTION #25  
300 fmol Injection - BSA Digest



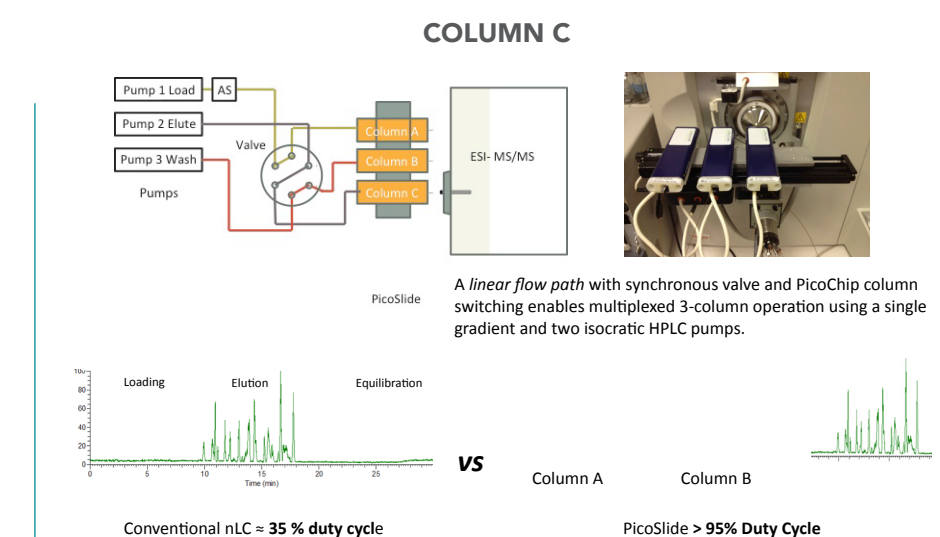
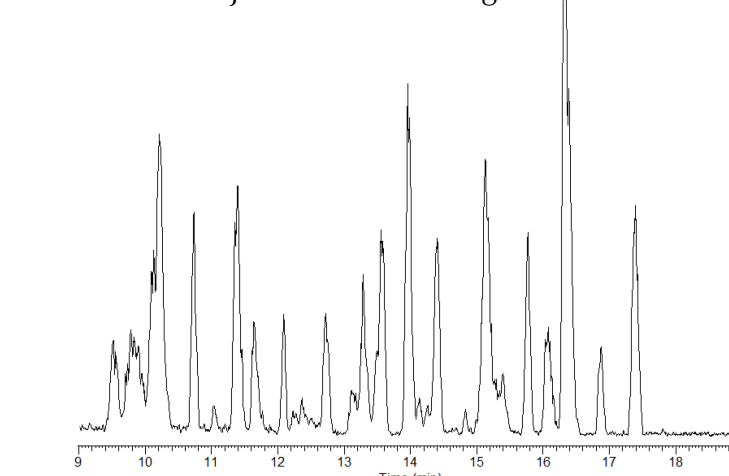
Conventional nLC = 35 % duty cycle VS PicoSlide > 95% Duty Cycle

Collect data from each column only during elution. While Column B is eluting analyte, Column C is loading sample, and Column A is equilibrating for the next injection cycle.

NL: 7.05E7  
INJECTION #5  
300 fmol Injection - BSA Digest



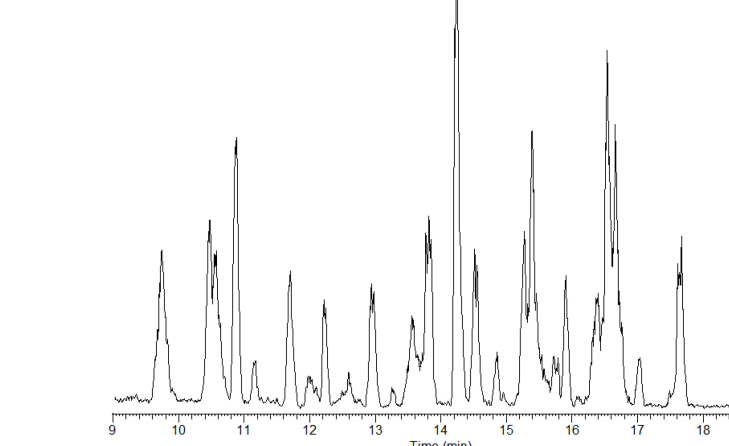
NL: 7.26E7  
INJECTION #25  
300 fmol Injection - BSA Digest



Conventional nLC = 35 % duty cycle VS PicoSlide > 95% Duty Cycle

Collect data from each column only during elution. While Column C is eluting analyte, Column A is loading sample, and Column B is equilibrating for the next injection cycle.

NL: 6.35E7  
INJECTION #5  
300 fmol injection - BSA Digest



NL: 6.43E7  
INJECTION #25  
300 fmol injection - BSA Digest

