

# Automated Nanospray Chip System for Medium Throughput Mass Spectrometry

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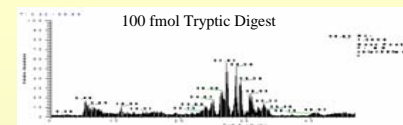
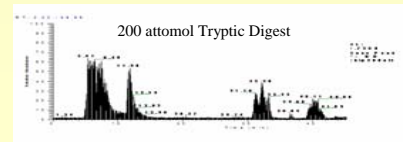
## Overview

Nanospray mass spectrometry has become an increasingly important technique for demanding applications such as ligand-native protein binding protein separation and identification using off-line LC-MS. In clinical diagnostics, the use of mass spectrometry has spread beyond the initial application of newborn genetic disorder screening to include disease diagnostics for cancers. The key to boost productivity of these applications where sample consumption must be kept to a minimum and reliability of the spray is critical is a nanospray system that is miniaturized, clog-resistant, and accurate with consistent flow and spray characteristics.

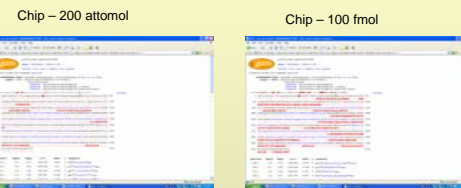
For these applications where the number of samples to be analyzed per day is in the hundreds, a chip with 16 samples may be sufficient. However, such a chip must be compatible with existing robotic sample dispensing equipment. The automated polypropylene-based nanospray chip may potentially meet all the requirements for productivity enhancement as well as cost-effectiveness.

## Sensitivity Enhancement by the Nanospray Chip

NanoLC- Chip-MS (75 mm x 5cm Biobasic C8)  
Digested Glutamate Dehydrogenase, Mobile phase:  
MeOH/H<sub>2</sub>O, MS: LTQ



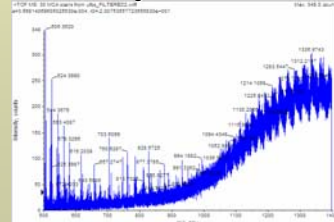
Search Engine Results based on MS i.d.



Mike Myers, Cold Spring Harbor Lab.

Non-clogging spraying of complex matrices:

Pre-centrifuged Fetal Bovine Serum



Tim Coleman, Correlogic Systems

## Nanospray nozzle and chip design



Nanospray chip : Nozzles side  
Nozzle spacing: 4.5 mm



Nanospray chip:  
nozzles and reservoirs

- Conical nozzle structures 0.5 mm to 3.5 mm in height
- 20+/- 3 μm i.d., 50 μm o.d.
- A reservoir of a few microliters to 15 microliters connects directly to each nozzle
- Four nozzles per chip; nozzles on 384-microtiter plate spacing
- Chips can be tiled to any configuration, up to a 384 microtiter-plate format

## Nanospray chip for medium throughput applications: 16-well Nozzle Chip



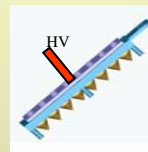
Nozzle side



Reservoir side

Four chips are tiled into a 16-nozzle configuration. To increase throughput, chips can be sequentially placed into the nozzle deploying interface station, or tiled into a 384-well microtiter plate.

Sealing mat/Diaphragm pump



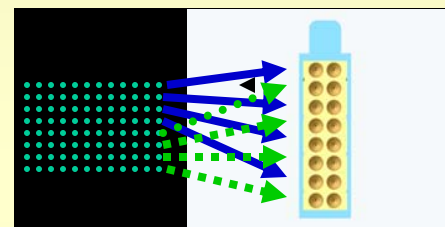
The preloaded sample is pumped out of the nozzle by pressing the plunger on the sealing mat acting as the diaphragm. During pumping, a high voltage is conveyed to the sample stored in the gold-coated reservoir by a contact pin mounted next to the plunger. Or if a special sealing mat with a conducting core is used, the metallic plunger carries the high voltage which goes through the mat into the sample. A cone-jet spray results, as shown in the adjacent panel. The flow rate can be regulated by changing the speed of the plunger. To calibrate the flow rates, a known amount of sample is loaded into the reservoir and the time it takes to empty the reservoir is measured for a particular speed of the plunger. The lowest flow rate we have achieved so far is 100 nL/min. Lower flow rates may be obtained if there is a layer of air between the mat and the sample. Pressing on the mat compresses the trapped air which creates a small pumping pressure.

## Interface for chip automation

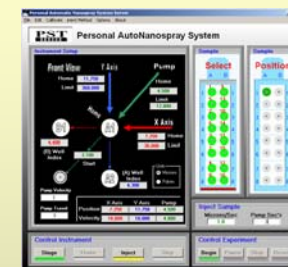


The chip and the pumping mechanism are mounted on a 3D positioner made of motorized stages with submicron accuracy.

## Adapting the 16-nozzle chip to a 96-well microtiter plate



## Restoring the sample order in the microtiter plate by spraying the samples in the microtiter plate order

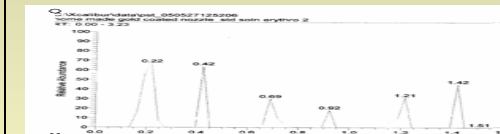


Nanospray by pumping sample from a reservoir sealed by a silicone mat with a conducting core



## Results

High speed nanospray of erythromycin in MeOH, 5 KV



Mass spectrum of a single scan (1s/scan, 200-1500 amu)



- Six samples in 90 s
- Good signal/noise for analyte for a single scan
- 2-3 s sample switching time possible
- Variation in intensities from sample to sample will be eliminated by a close-loop control of spray current (under development).

## Summary

- A new plastic nanospray chip/plate with automated operation is presented
- By preloading the sample into the reservoir behind each nozzle, the dead time for switching the spray from nozzle to nozzle can be reduced to ~1 second.
- The chip may be adapted to conventional sample dispensing robotics for 96-well or 384-well plates.
- The pumping mechanism is based on a plunger/diaphragm arrangement. Flow rates lower than 100 nL/min. will be achievable.
- Preliminary results indicated <10 s/sample will be possible with the automated nanospray plate.
- Numerous applications from drug discovery to clinical diagnostics may benefit from medium throughput mass spectrometry using the automated nanospray plate.

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