

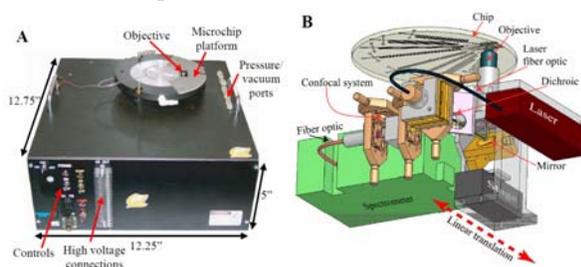
## Multichannel Mars Organic Analyzer (McMOA): Microfluidic Networks for the Automated *in Situ* Microchip Electrophoretic Analysis of Organic Biomarkers on Mars.

Thomas N. Chiesl, Merwan Benhabib, Amanda M. Stockton, Richard A. Mathies, Department of Chemistry, University of California, Berkeley, CA 94720, ramathies@berkeley.edu

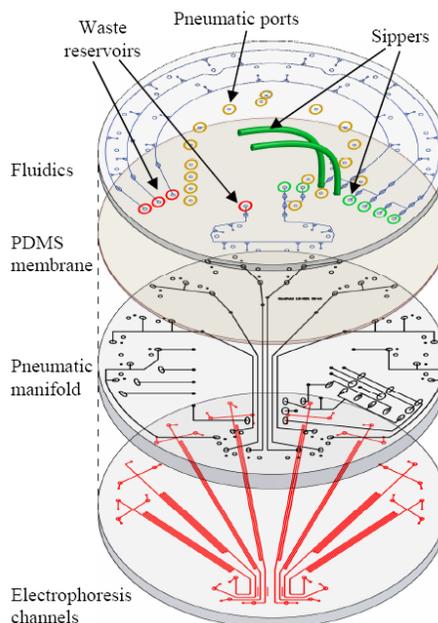
### Introduction:

Previously, our group created the Mars Organic Analyzer (MOA), a single channel portable instrument that was successfully field tested in the Panoche Valley, CA, and in the Atacama Desert, Chile, where it detected trace amines and amino acids using fluorescamine as the derivatization reagent.<sup>1-2</sup> Recent work has extended the MOA's capabilities to perform analysis of polycyclic aromatic hydrocarbons (PAHs) at ppb levels.<sup>3</sup> Through use of a highly fluorescent amine reactive probe, Pacific Blue Succinimidy Ester, PB, our detection limits for amino acids have improved 250-fold.<sup>4</sup> Furthermore, the integration of PB labeling with micellar electrokinetic chromatography (MEKC) enables pseudo-2D separations, allowing for enhanced compositional analysis.<sup>4</sup> Finally, recent advances in analytical protocols and buffer chemistry have improved the robustness of our CE systems and methods when analyzing highly saline and acidic samples as found in Rio Tinto sediments and Saline Valley brine samples.<sup>5</sup>

Here, we present the next generation Multichannel Mars Organic Analyzer (McMOA),<sup>6</sup> as seen in Figure 1. The instrument uses a four-layer microchip containing eight CE analysis systems integrated with a microfluidic network for fluidic processing (Figure 2). The goal of this work is to achieve autonomous routing and analysis of samples on redundant CE microchannels with negligible sample carry-over. The development of microfluidic architectures that integrate automated metering and mixing of fluids for performing on chip reactions and serial dilutions is also discussed. Here we present the design and operation of a multichannel chip and instrument system to address these goals and evaluate its performance.



**Figure 1.** A) Multichannel Mars Organic Analyzer Instrument. B) SolidWorks rendition of the optical system showing the laser with fiber optic, optical adjustments, dichroic lens, and the stage that holds the mirror and objective.

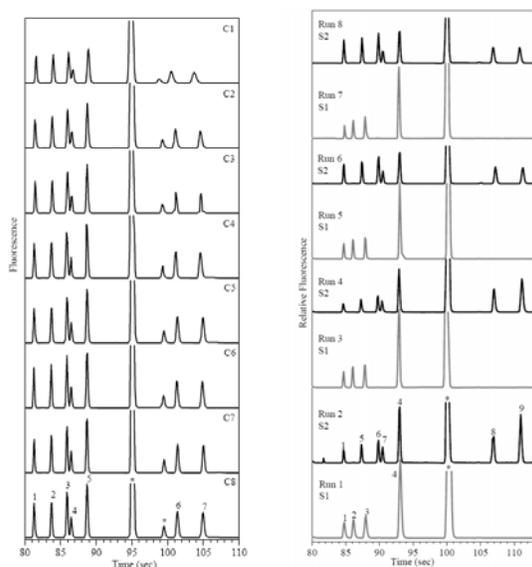


**Figure 2.** Microfabricated device assembly in exploded view. Waste reservoirs, pneumatic ports and sippers to external buffer or sample reservoirs are accented with red, orange, and green circles, respectively. Two sippers are provided for each electrophoresis reservoir.

### Results:

The McMOA, pictured in Figure 1, was developed to operate 8 independent electrophoresis channels integrated with a microfluidic network for sample introduction, routing and analysis. The McMOA includes a linear scanning optical excitation system, a charged-coupled device (CCD) fluorescence spectrometer detection system and a temperature controlled chip platform. The system was found to have a 6 pM instrumental limit of detection.

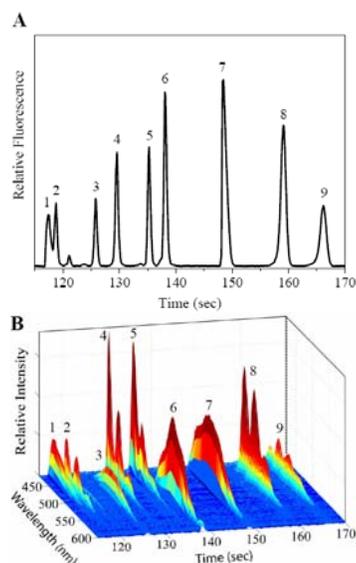
Figure 3 presents electropherograms obtained from the autonomous operation of a McMOA program designed to load buffer and sample sequentially to each of the 8-channels, perform a separation, and then empty the channels. The similarity in results and quality of separations performed on separate channels confirms the success of our design in establishing multichannel redundancy and automated integrated microfluidic access.



**Figure 3.** (Left) Electrophoretic separations of an amino acid standard in 4 mM  $\text{Li}_2\text{CO}_3$  (pH 8.5) buffer at room temperature and 700 V/cm on the McMOA. The sample was automatically routed from the sample reservoir to each channel (C1 to C8) sequentially and analyzed. (Right) Electropherograms from eight consecutive automated loading and analyses of two alternating samples (S1 and S2) in channel 6. An automated rinse step with buffer was performed between each analysis.

A series of experiments were performed to measure sample-to-sample carryover when a CE channel system is reused. Figure 3 presents electropherograms from eight autonomous separations performed on channel 6 where the sample analyzed alternates between a concentrated sample and a dilute sample. By examining the peak areas, it was found that less than 1 % of leucine and threonine were carried over from the higher concentrated S1 to the consecutive separation and less than 0.7 % of valine, serine, alanine, glutamic acid and aspartic acid were carried over from the less concentrated S2 to the consecutive separation.

The CCD spectrometer was used to identify the unique fluorescence spectra of nine components in a PAH standard and then applied to the analysis of a sediment sample from Lake Erie. Figure 4A shows the conventional electropherogram of fluorescence integrated over all wavelengths (from 420 to 600 nm) versus time obtained using the McMOA to analyze a standard containing nine PAHs. The concentration of PAHs used in this separation range from 100 nM for perylene to 100  $\mu\text{M}$  for anthracene. Figure 4B presents a 3D plot of the same separation but with the fluorescence displayed as a function of the emission wavelength and separation time. This analysis method allows for unambiguous identification of PAHs that might co-elute.



**Figure 4.** Real-time spectral analysis and separation of PAHs on the McMOA. (A) Electrophoretic separation with standard optical detection and integration of emission between 420 and 600 nm. (B) McMOA operating in real-time 3D fluorescence spectroscopic analysis mode. Sample contains: (1) 5  $\mu\text{M}$  diphenylanthracene, (2) 20  $\mu\text{M}$  dibenzo[b,def]chrysene, (3) 100  $\mu\text{M}$  anthracene (4) 400 nM anthanthrene, (5) 4  $\mu\text{M}$  benzo[a]pyrene, (6) 50  $\mu\text{M}$  benzo[j]fluoranthene, (7) 500  $\mu\text{M}$  fluoranthene, (8) 100 nM perylene, (9) 50  $\mu\text{M}$  benzo[ghi]fluoranthene.

**Conclusions:** We have successfully built and tested a next generation *in situ* planetary exploration instrumentation for organic compound analysis. The McMOA exploits lab-on-a-chip technologies to fully integrate complex autonomous operations demonstrating the facile engineering of microchip-CE platforms for the analysis of a wide variety of organic compounds in planetary exploration.

**Additional Information:** For more information on the MOA and McMOA, see <http://astrobiology.berkeley.edu>

#### References

- (1) Skelley, A. M.; Scherer, J. R.; Aubrey, A. D.; Grover, W. H.; Ivester, R. H. C.; Ehrenfreund, P.; Grunthner, F. J.; Bada, J. L.; Mathies, R. A. *Proc. Natl. Acad. Sci U.S.A.* **2005**, *102*, 1041-1046.
- (2) Skelley, A. M.; Aubrey, A. D.; Willis, P. A.; Amashukeli, X.; Ehrenfreund, P.; Bada, J. L.; Grunthner, F. J.; Mathies, R. A. *J. Geophys. Res.* **2007**, *112*.
- (3) Stockton, A. M.; Chiesl, T. N.; Scherer, J. R.; Mathies, R. A. *Analytical Chemistry* **2009**, *81*, 790-796.
- (4) Chiesl, T. N.; Chu, W. K.; Stockton, A. M.; Amashukeli, X.; Grunthner, F.; Mathies, R. A. *Anal. Chem.* **2009**, *81*, 2537-2544.
- (5) Stockton, A. M.; Chiesl, T. N.; Lowenstein, T. K.; Amashukeli, X.; Grunthner, F.; Mathies, R. A. *Anal. Chem.* **2009**.
- (6) Benhabib, M.; Chiesl, T. N.; Stockton, A. M.; Scherer, J. R.; Mathies, R. A. *Analytical Chemistry* **2009**, Submitted.