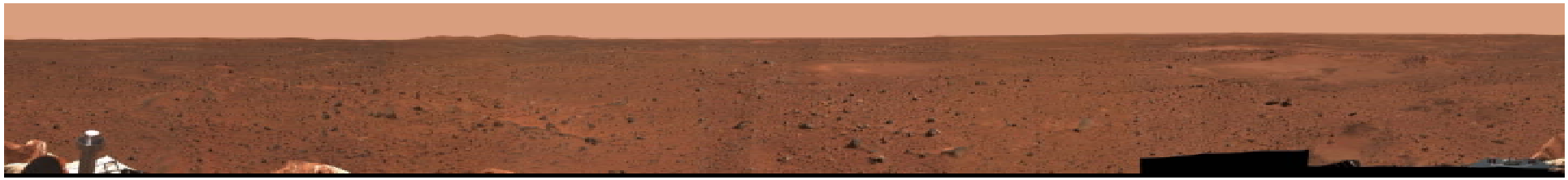


Microfabricated Organic Analyzer (MOA) for *in situ* Exploration of Mars and other Solar Bodies

- Prof. Richard A. Mathies, Chemistry Department, UC Berkeley, microfabricated devices and biochemical analysis

- Prof. Jeff Bada, Scripps Institution of Oceanography, UCSD, amino acid analysis and astrobiology

- Dr. Frank Grunthaner, Jet Propulsion Laboratory, instrument design, operation and flight engineering

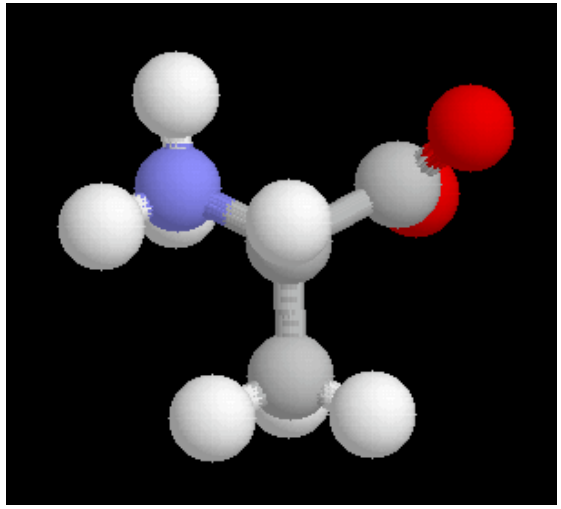
Project Goals

- Complete brass-board development of microfabricated capillary electrophoresis (CE) chip and instrument for amino acid analysis
- Integrate the microchip CE system with MOD sampling system to form the Mars Organic Analyzer (MOA)
- Perform field tests of the MOA in Mojave and three Mars-like Atacama sites
- Document maturation, integration and field operation of two MIDP, PIDDP and ASTID-derived instruments
- Enhance TRL of MOA through Mojave and Atacama field testing
- Increase our understanding of limits and constraints of life in extreme environments
- Critically define identity of and sensitivity requirements of potential biomarkers.

Amino Acid Composition and Chirality Analysis

Potential bioorganic signatures:

- **Large biomolecules likely degraded by oxidizing surface environment of Mars**
- **Amino acids have a longer lifetimes in dry, harsh conditions**
- **Amino acids have been found in meteorites**
- **Amino acid chirality is indicative of origin:**
  - **Racemic mixture – abiotic origin**
  - **Non-racemic mixture – biological origin**



L-alanine

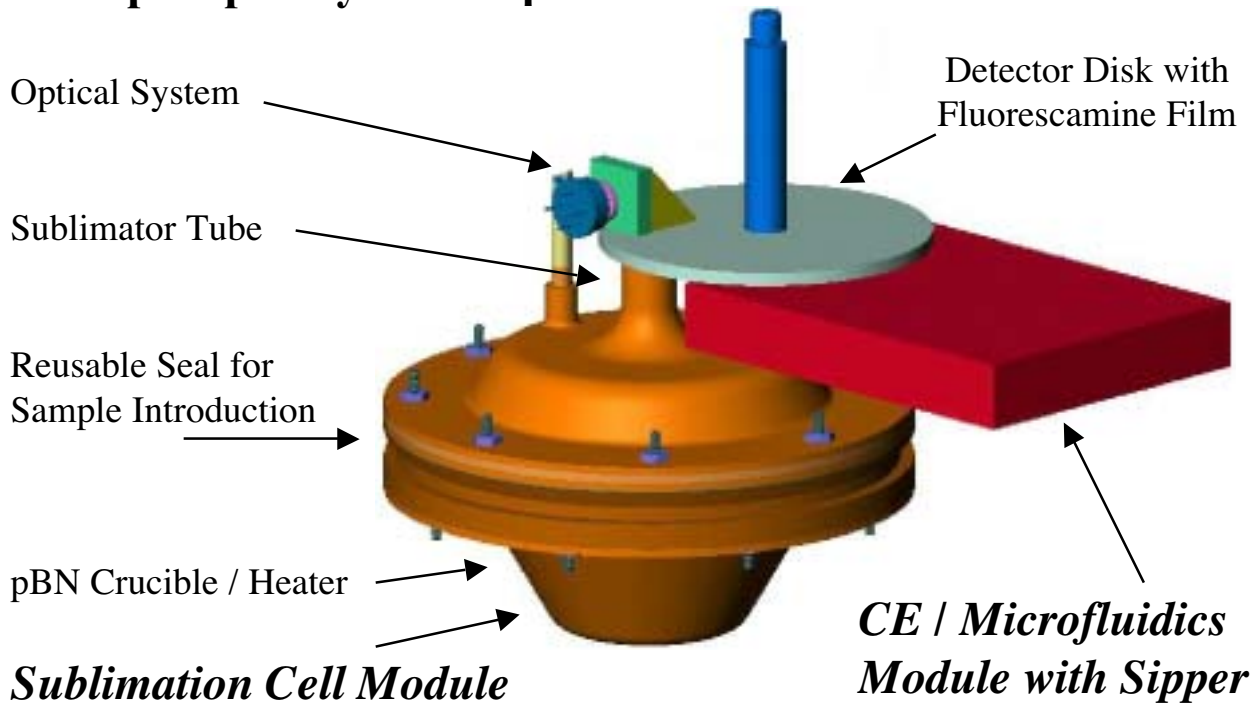
Why *in situ* analysis:

- **Significant contamination of Meteorites found on earth by terrestrial sources of life**
- **Sample return missions are more technologically challenging, costly and time consuming**

Allan Hills Meteorite

Mars Organic Analyzer (MOA) Concept

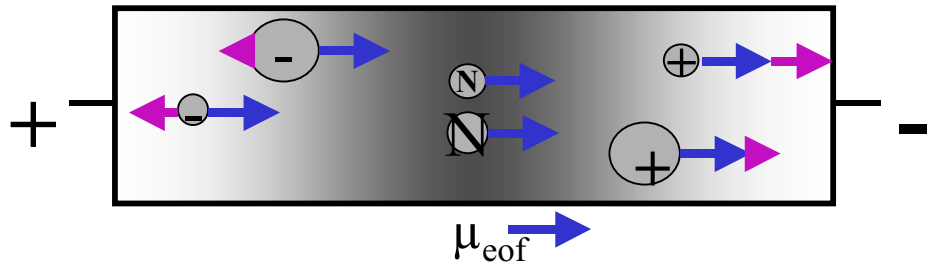
- **Soil samples collected and deposited into Mars Organic Detector (MOD)**
- **MOD sublimes amino acids onto cold finger coated with fluorescamine[1]**
- **Fluorescamine-labeled amino acids analyzed for composition and chirality via microchip Capillary Electrophoresis**



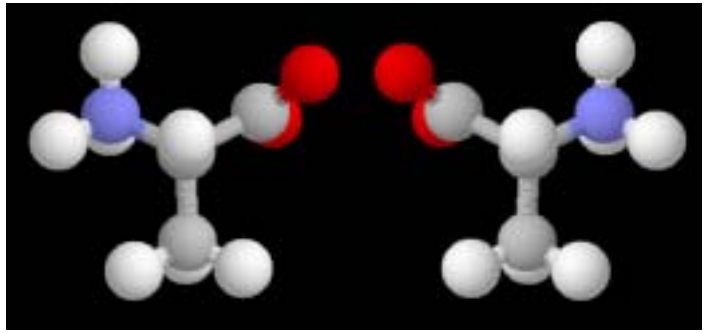
[1] Kminek, et al., *Planetary & Space Science* 2000, *48*, 1087-1091.

Composition and Chirality Analysis by CE

- **Electroosmotic flow (EOF) sweeps all molecules to the cathode**

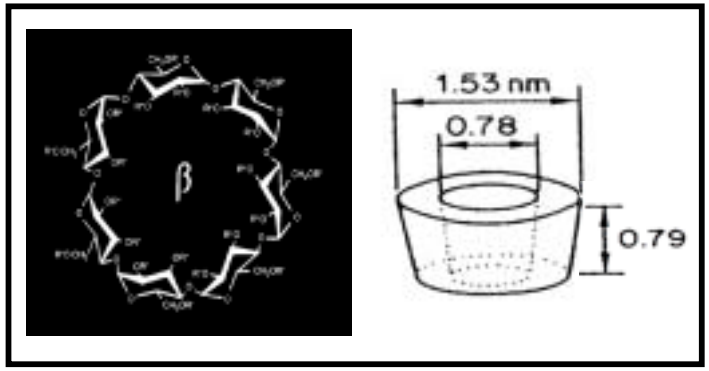


- **CE separates amino acids based on charge/size ratio giving composition information**



L-alanine **D-alanine**

- **Cyclodextrins included in running buffer provide enantiomeric resolution of amino acids**

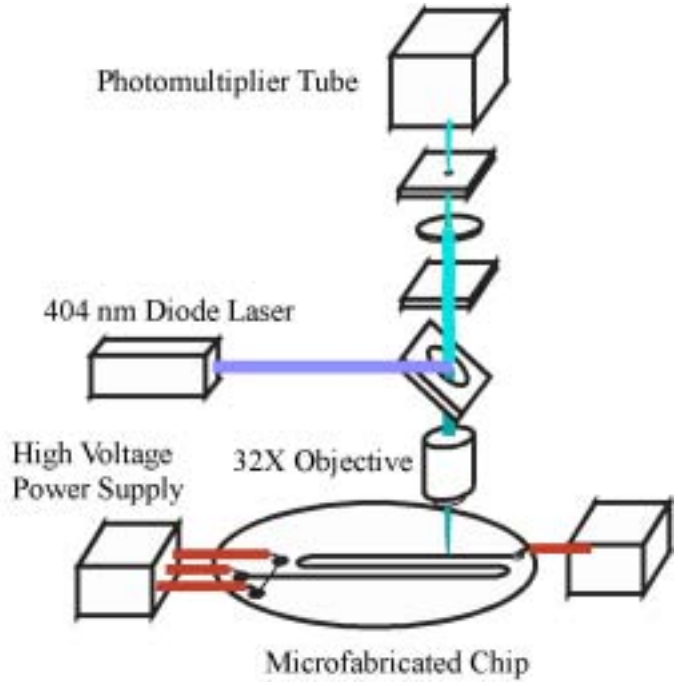


γ-cyclodextrin

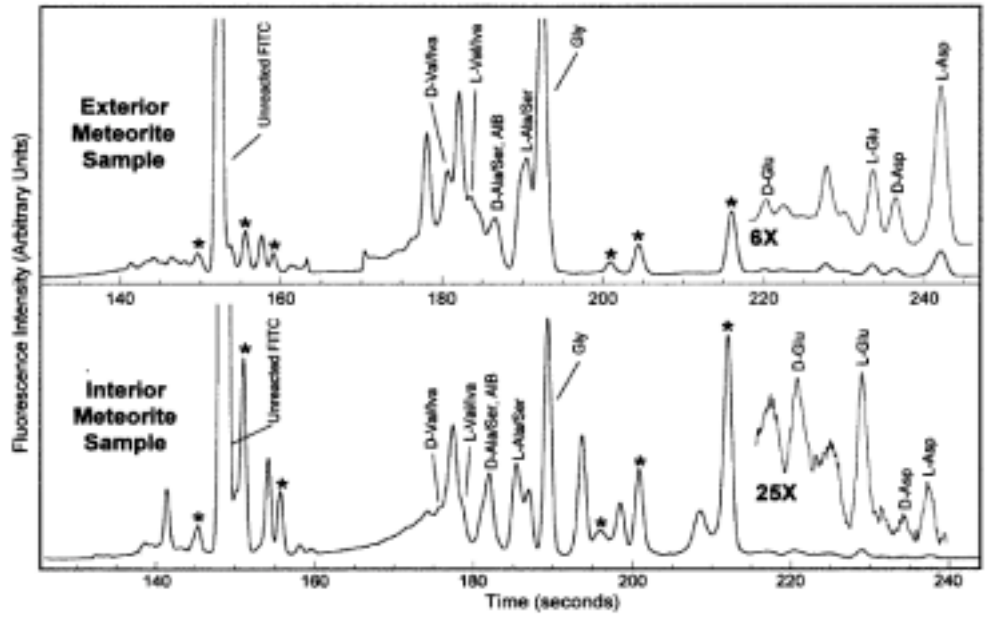
$$\text{L-aa} + \text{CD} \Rightarrow \text{L-complex} \ (K_L)$$
$$\text{D-aa} + \text{CD} \Rightarrow \text{D-complex} \ (K_D)$$

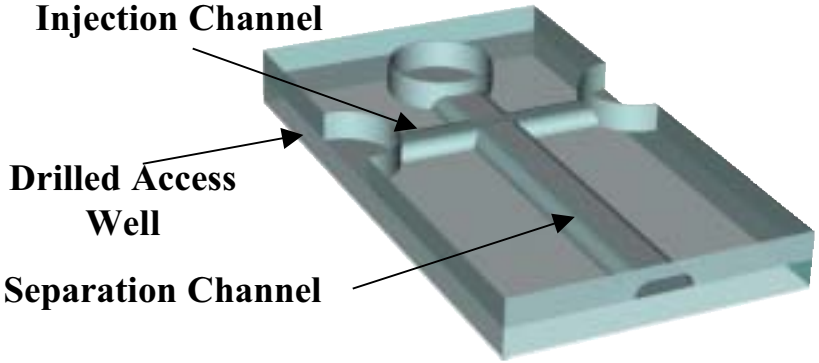
$$K_L \neq K_D$$

Established Separation and Lab-Based Detection System



Analysis of Fluorescein-labeled Amino Acids





Murchison Meteorite Glu and Asp D/L values

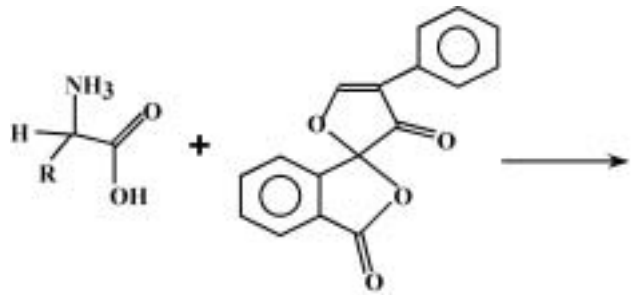
Amino Acid	HPLC	Microchip CE
Glu		
Exterior	0.3 + 0.1	0.33 + 0.04
Interior	0.7 + 0.1	0.65 + 0.07
Asp		
Exterior	0.3 + 0.1	0.21 + 0.03
Interior	0.3 + 0.1	0.30 + 0.06

Hutt, L. D.; Glavin, D. P.; Bada, J. L.; Mathies, R. A. *Analytical Chemistry* 1999, *71*, 4000-4006.

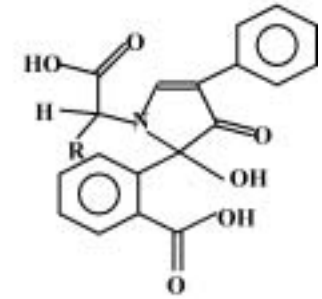
Separation of Fluorescamine-labeled Amino Acids

Advantages of Fluorescamine:

- **Fluorogenic reagent**
- **Reaction time ~1 min**
- **~ 50 nM LOD attainable**
- **Reagent used in MOD**

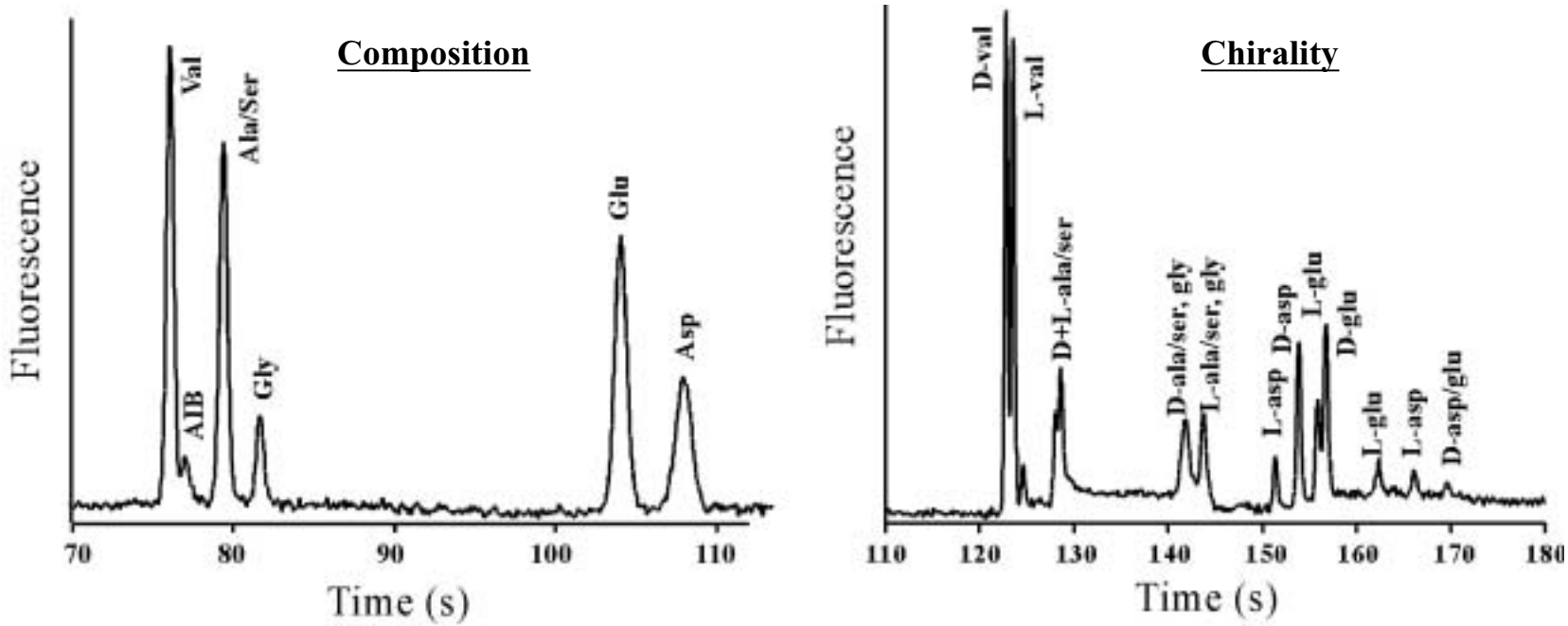


Fluorescamine (FA)



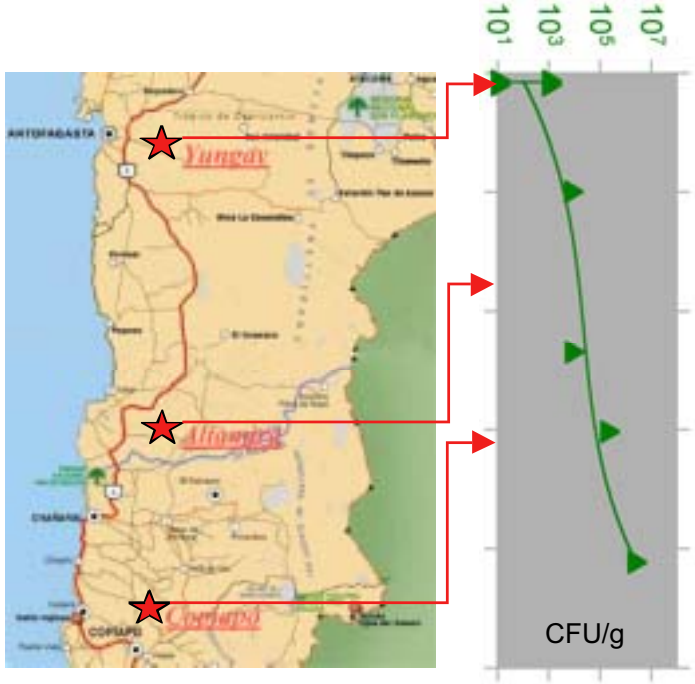
Derivatized amino acid (absorption max @ 390 nm)

Separation of Mars 7 Standard labeled with Fluorescamine



A. M. Skelley and R. A. Mathies, *J. Chromatogr. A* 2003, *1021*, 191-199.

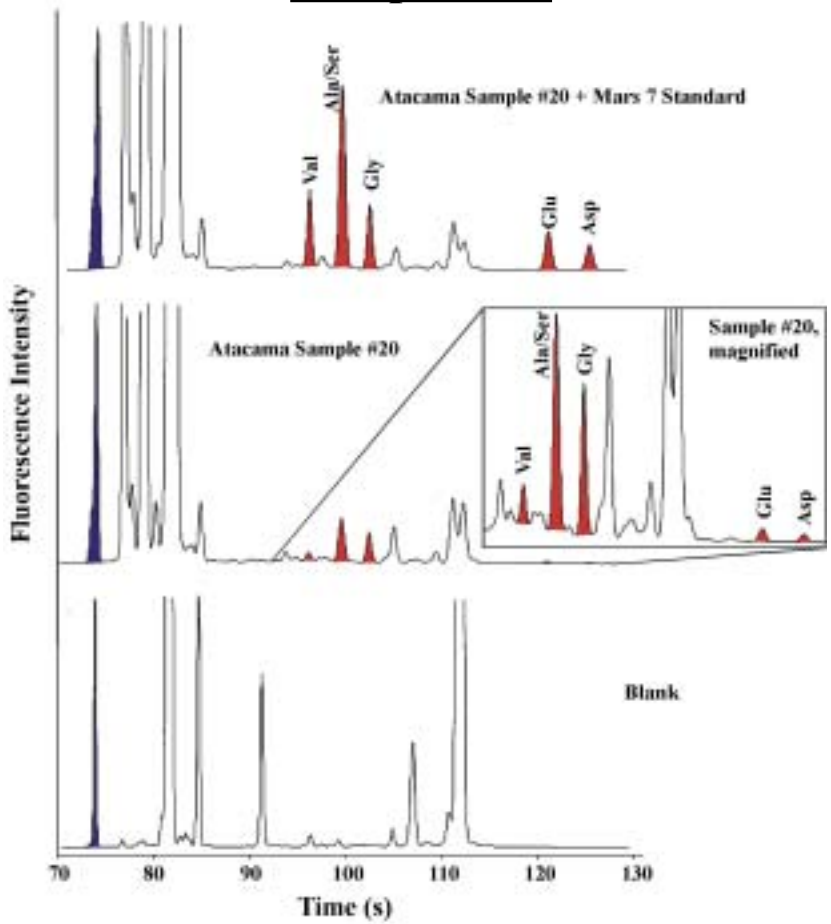
Atacama Desert as Martian Analog Site



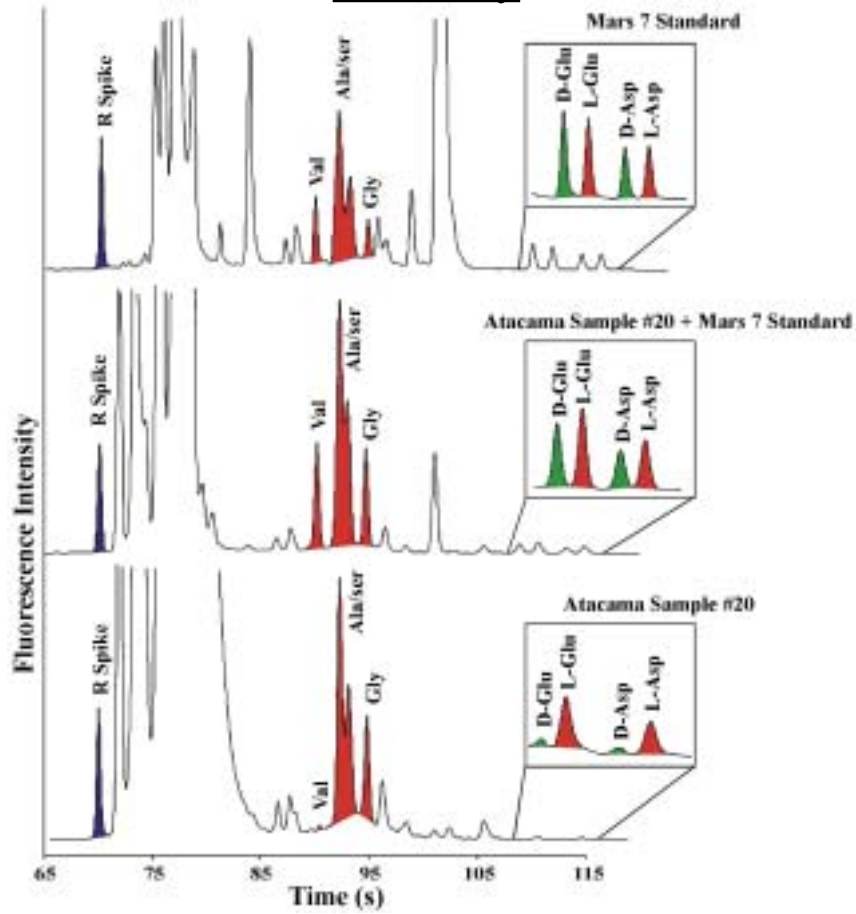
- **Chilean Atacama Desert is one of the driest sites on the planet (<0.5 mm H_2O/year).**
- **The transect from Lat 24˚ to 28˚ South at 69.5˚ West has been extensively studied.**
- **Some areas have unusual surface oxidation chemistry and organic soil concentrations at lab blank levels. Other areas show readily detected microbial and higher life forms.**

Analysis of Atacama Soil Extracts

Composition



Chirality



Blank-corrected Concentrations (weight/weight):

Val = 0.034 ± 0.009 ppm	Ala/ser = 0.32 ± 0.07 ppm
Gly = 0.18 ± 0.03 ppm	Glu = 0.14 ± 0.02 ppm
Asp = 0.094 ± 0.004 ppm	

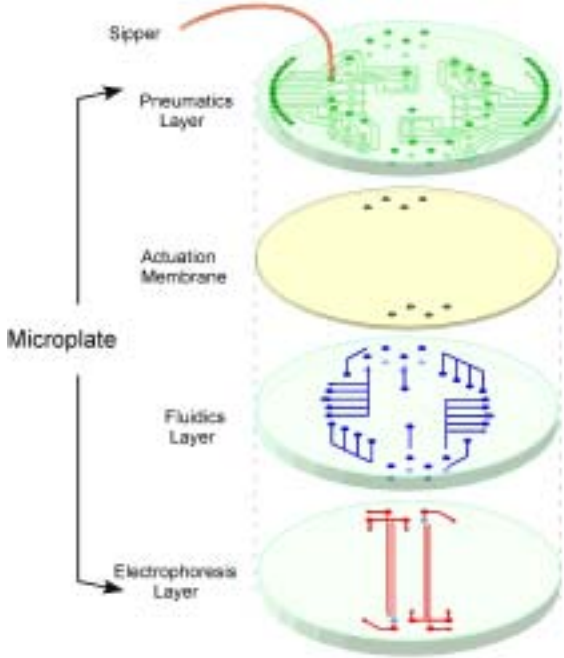
Blank-corrected Enantiomeric Ratios:

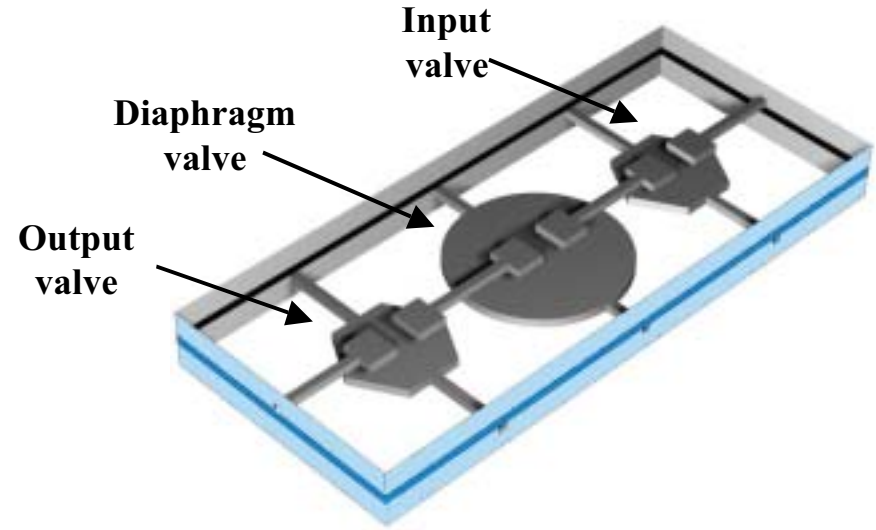
Mars 7 Standard:	Atacama Sample # 20
D/L Glu = 1.10 ± 0.02	D/L Glu = 0.22 ± 0.02
D/L Asp = 0.97 ± 0.02	D/L Asp = 0.16 ± 0.02

Berkeley, UCSD and JPL

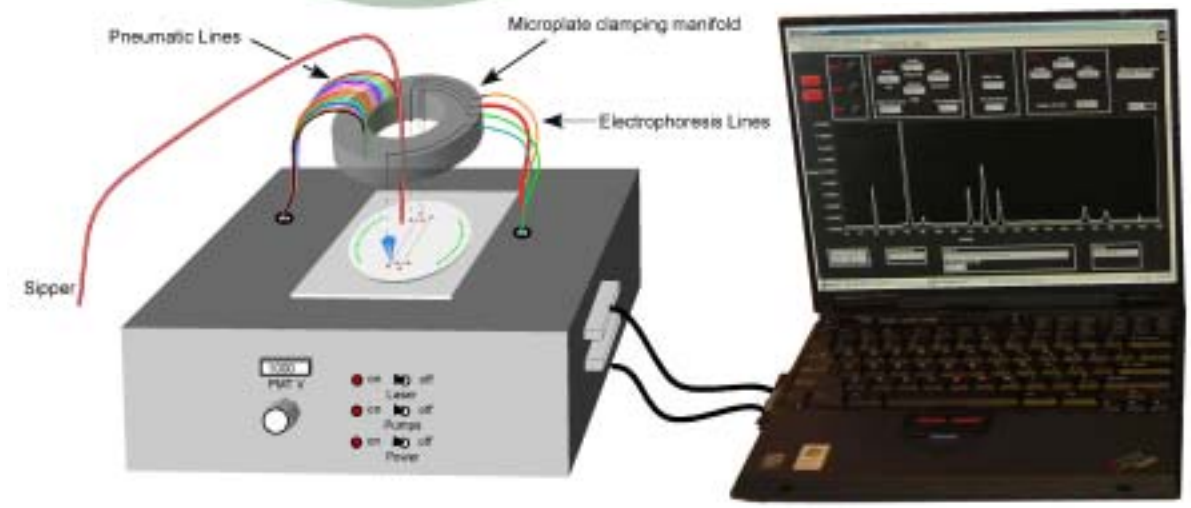
Mars Organic Analyzer (MOA)

Portable Microchip CE System - Schematic





W.H. Grover, A.M. Skelley, C.N. Liu, E.T. Lagally, R.A. Mathies, *Sens. Actuators B* 89 (2003) 325.



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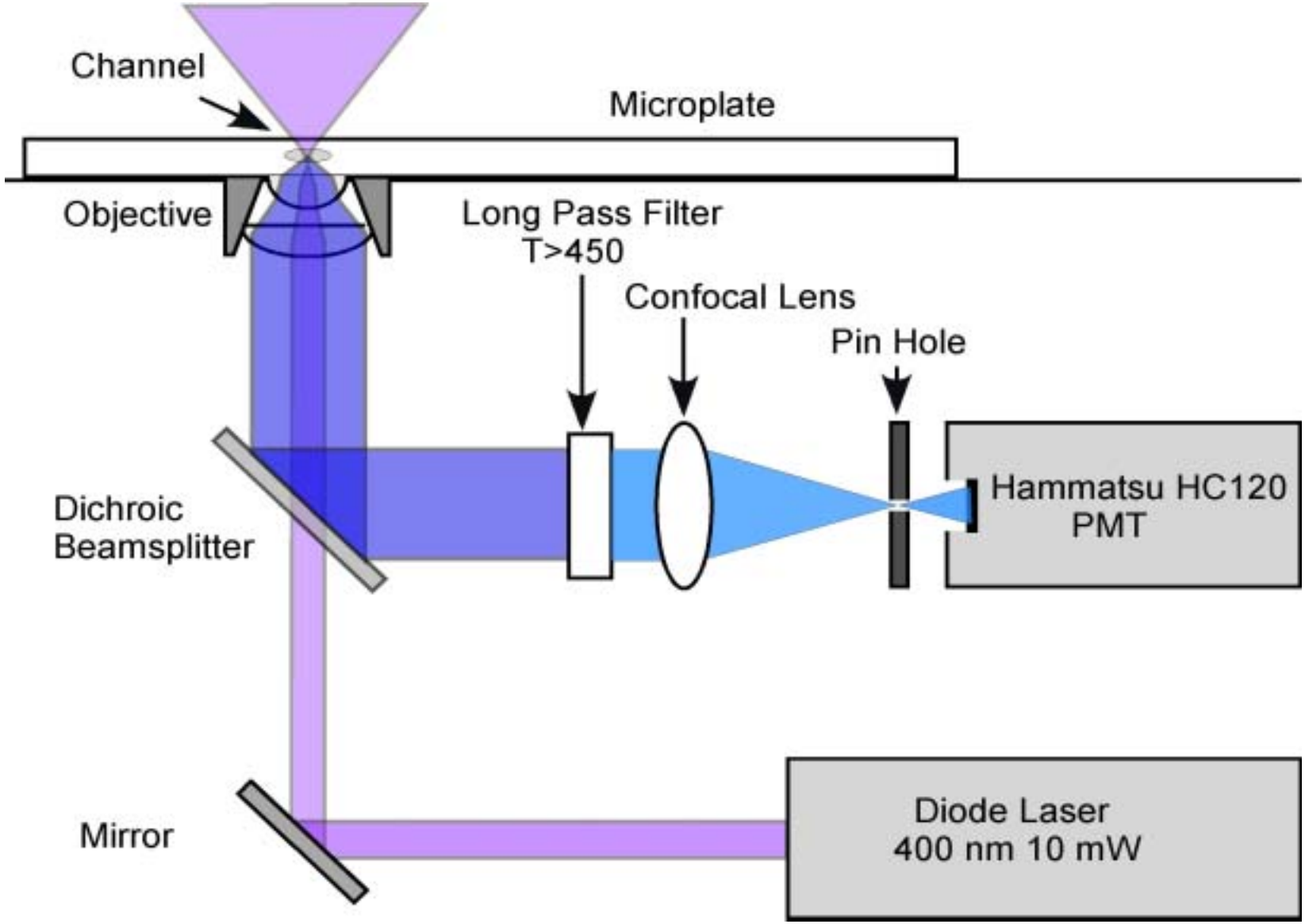
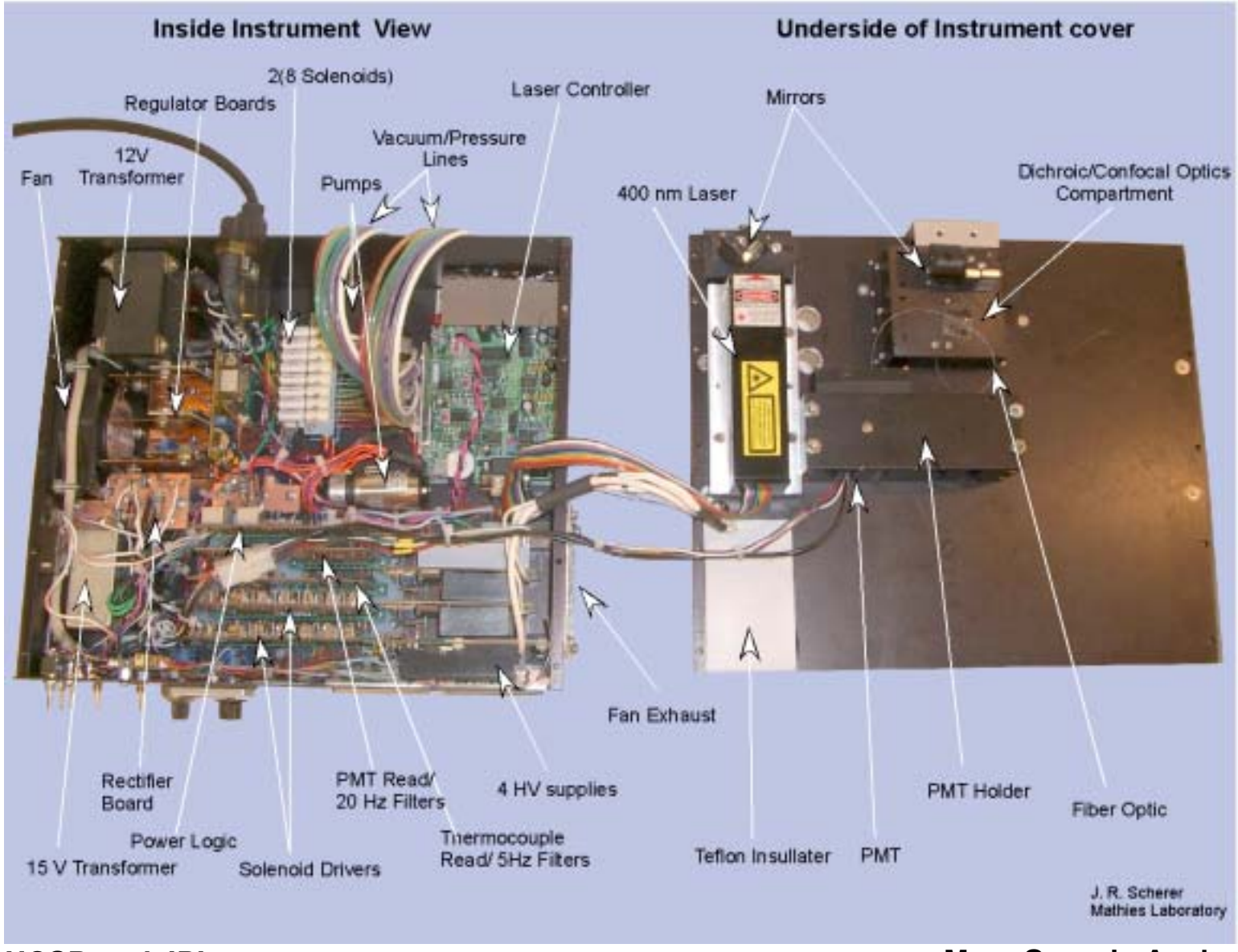
Mars Organic Analyzer (MOA)

Portable Microchip CE Instrument



Portable Microchip CE Instrument



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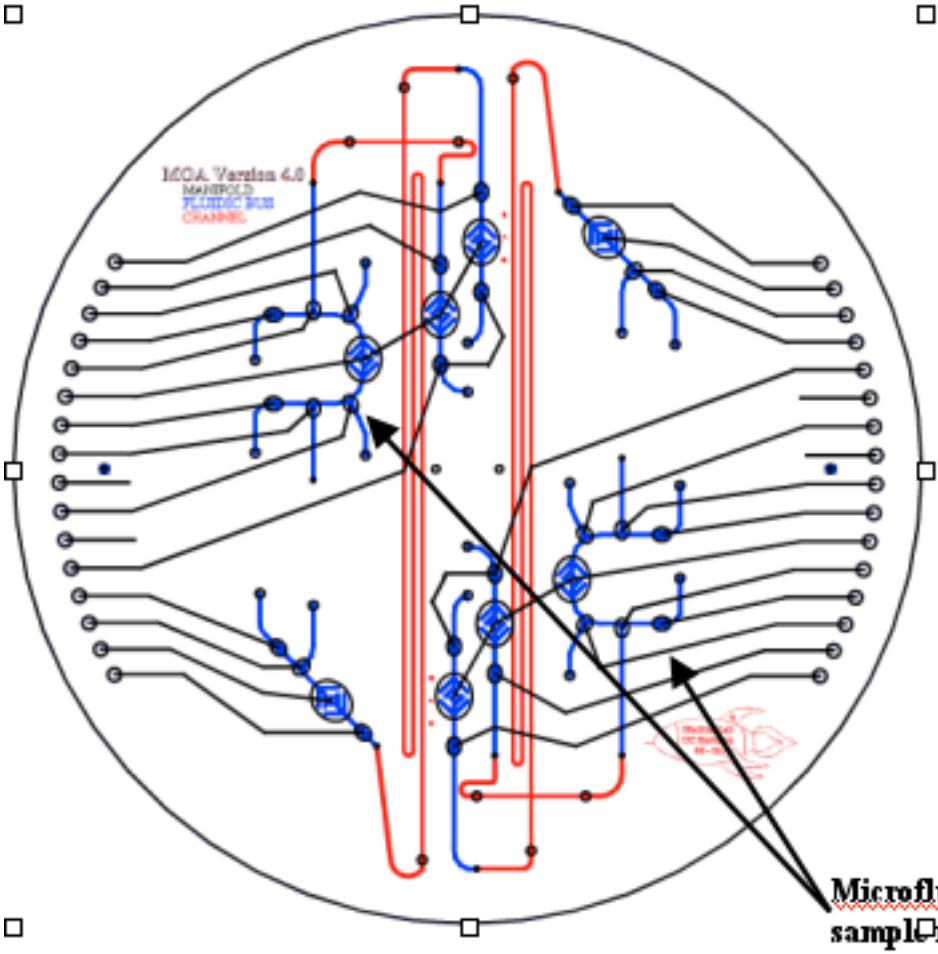
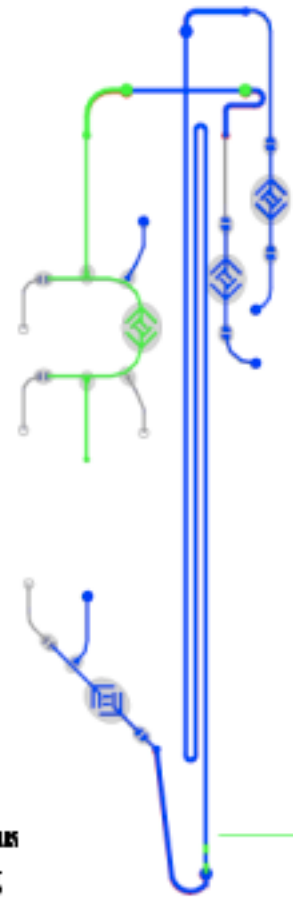
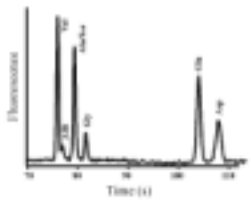
Mars Organic Analyzer (MOA)

Microfabricated Device



Microfluidic bus sample routing





Electropherogram

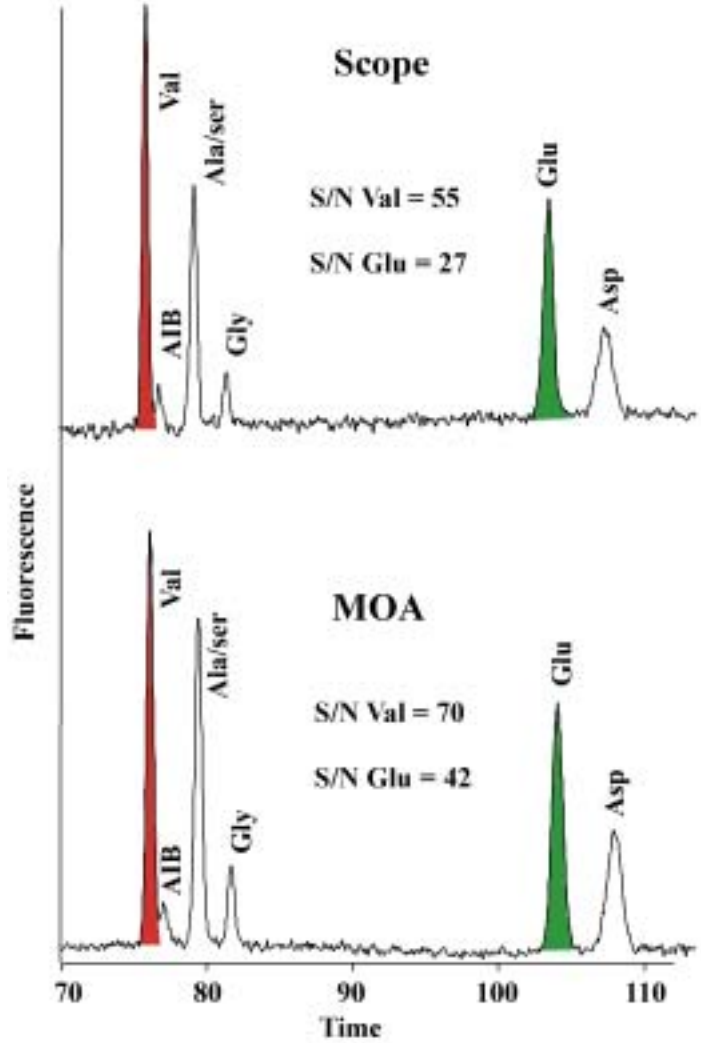
Fluorescence detection

- 4-layer structure, < 4 mm thick
- Diaphragm pumps deliver ~ 1 uL per actuation
- Input/output valves can actuate in under 100 ms
- Flow rates up to 350 nL/s are obtained

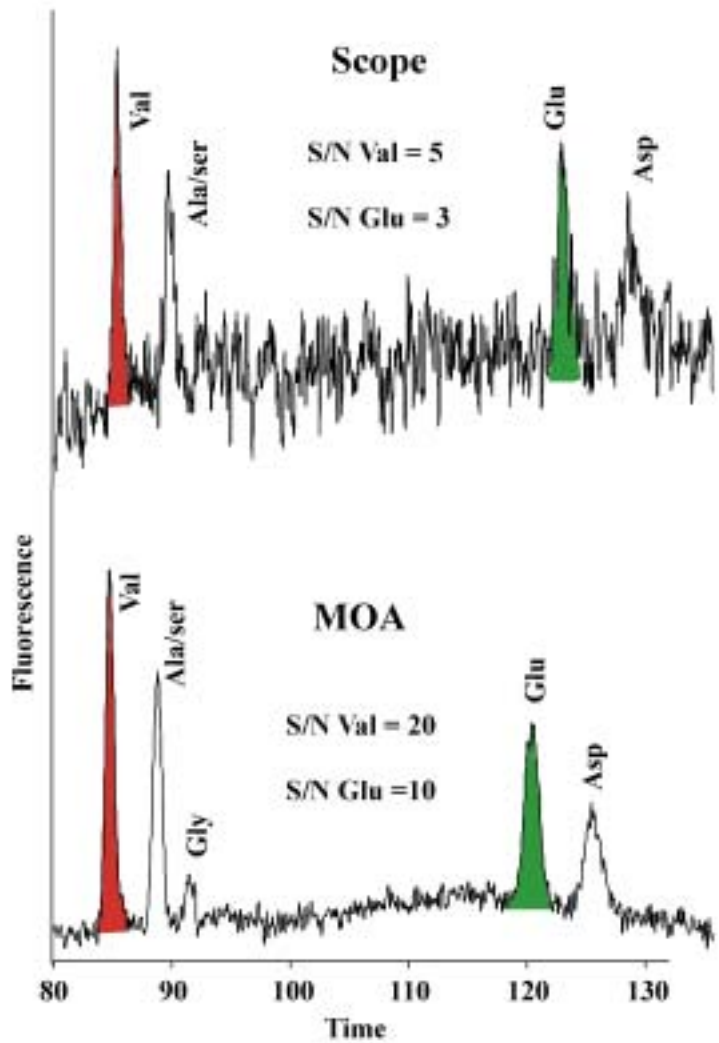
- Microfluidic bus has 5 reservoirs for buffer, water, labeling dye, waste, etc., reactor valve and sipper
- Rinsing bus allows device to be used for multiple samples
- 2 separation channels, 21 cm long

Comparison of Lab and MOA Systems

Concentration of each AA = 2.2 µM



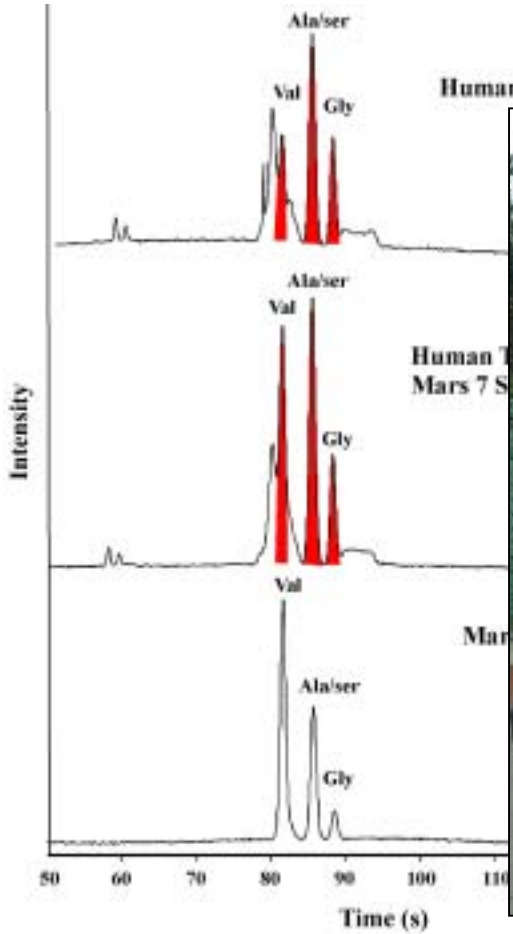
Concentration of each AA = 444 nM



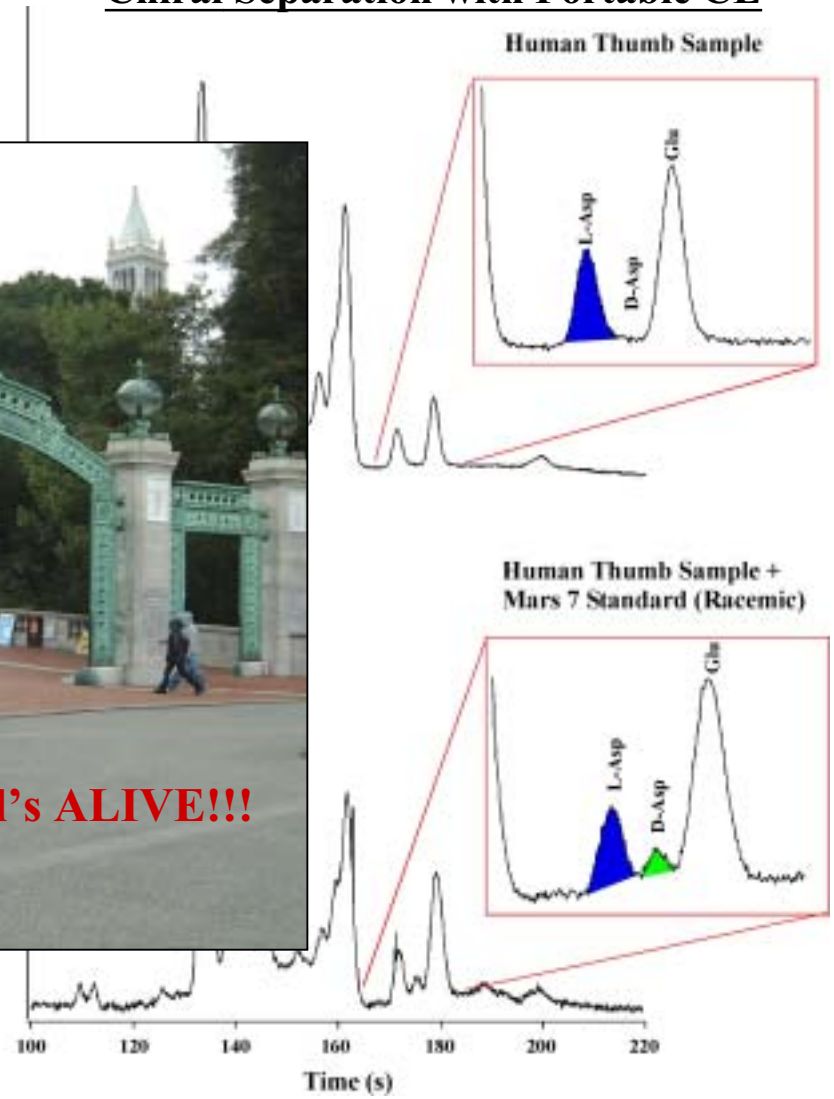
•MOA system shows superior sensitivity and comparable separation efficiency

Life in Berkeley?

Achiral Separation with Portable CE



Chiral Separation with Portable CE



Amino acid concentration in 50 µL collected sample:

Val = 16.2 Ala/Ser = 92.1 Gly = 622.0 µM
Glu = 11.7 Asp = 16.3

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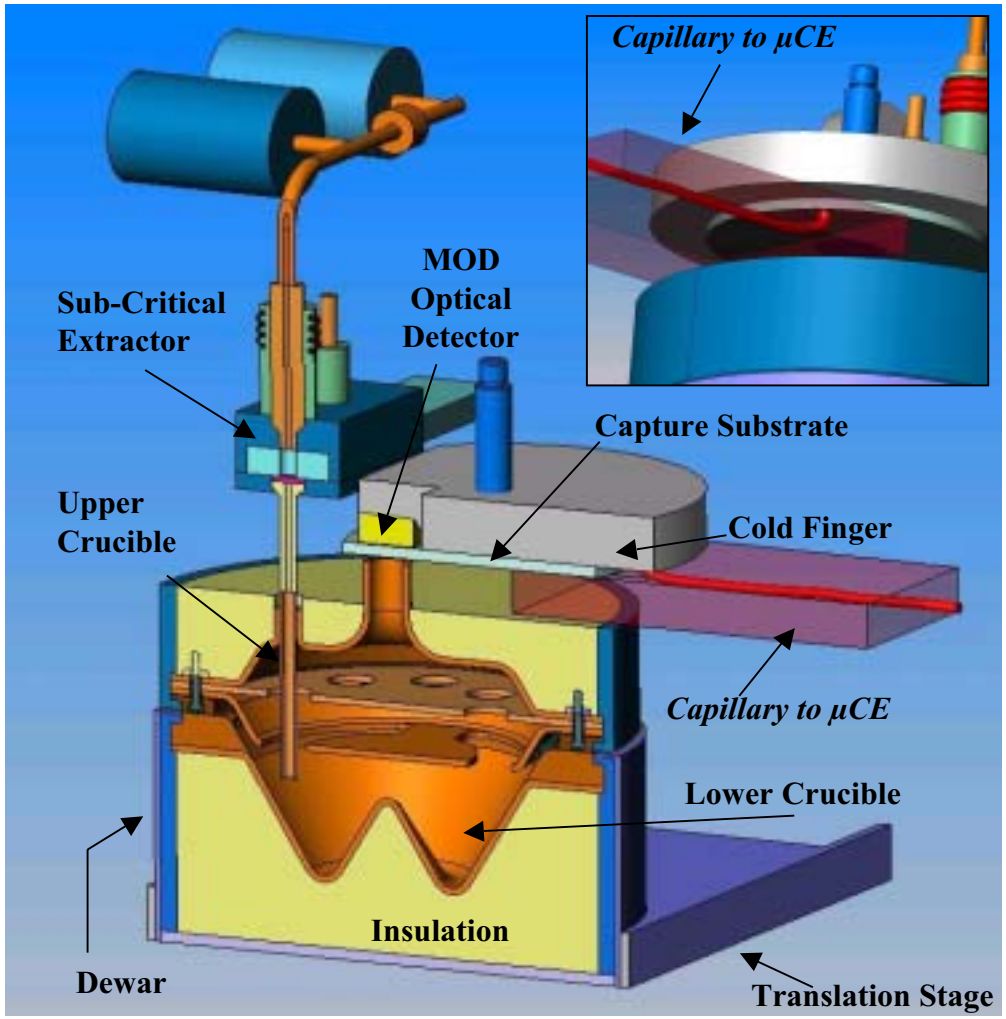
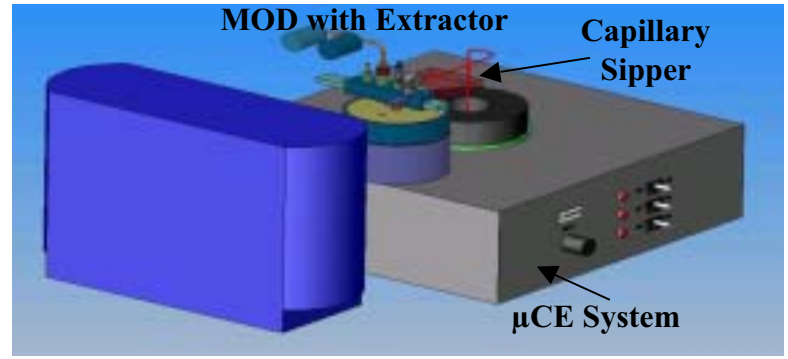
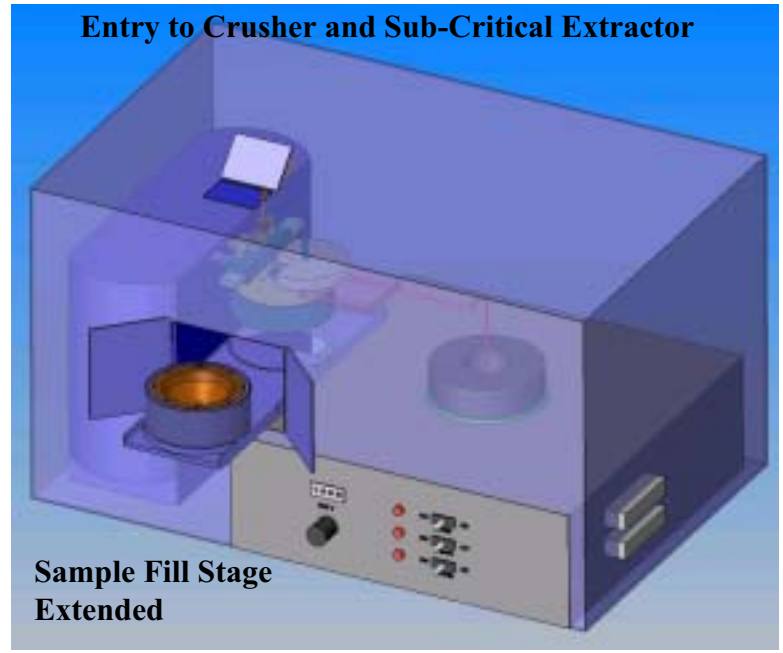
Mars Organic Analyzer (MOA)

MOD + CE = MOA


Capillary to µCE
Sub-Critical Extractor
MOD Optical Detector
Capture Substrate
Upper Crucible
Cold Finger
Capillary to µCE
Lower Crucible
Insulation
Dewar
Translation Stage


MOD with Extractor
Capillary Sipper
µCE System


Entry to Crusher and Sub-Critical Extractor
Sample Fill Stage Extended

Summary

- **Amino acid composition and chirality is an ideal means for organic biomarker detection on Mars.**

- **Microfabricated CE instrument provides a demonstrated means for sensitive amino acid composition and chirality analysis.**

- **Portable CE instrument has identical separation efficiency and greater sensitivity than standard lab systems.**

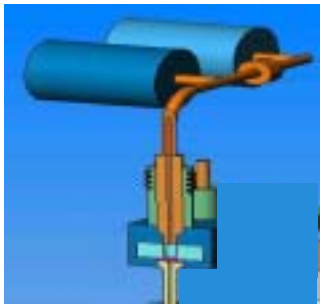
- **Integration of CE with MOD to make MOA will provide sensitive analysis of amino acids in Martian soil.**

- **Field tests in Mojave and Atacama Deserts are planned as a critical test of technology readiness and analysis capabilities.**

- **Microchip is a powerful platform for preparation other analytes from other sources for many types of analyses.**

Mars Organic Laboratory (MOL): Beyond amino acids

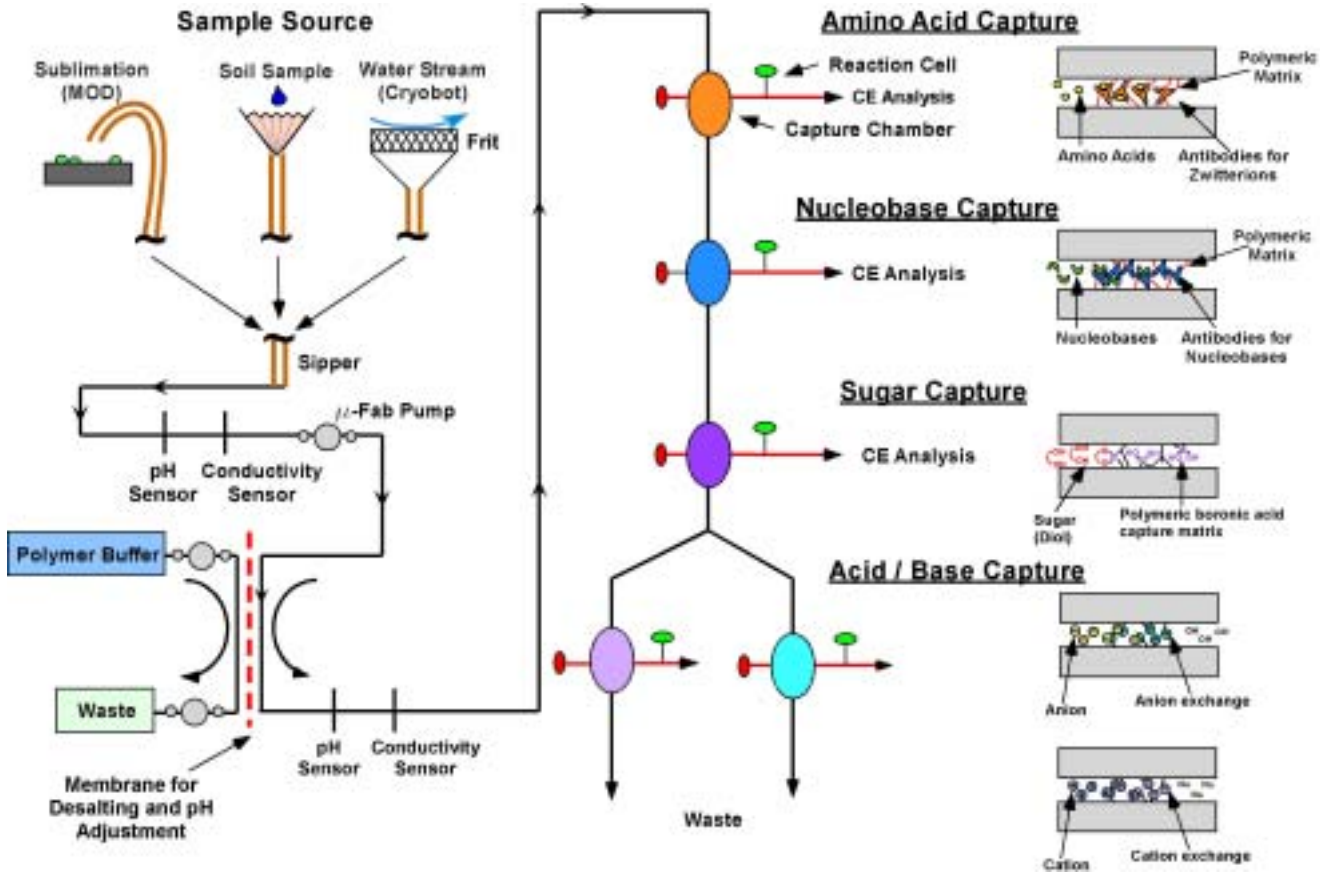
MOD



Sub-Critical Extractor

Cryobot

MOL Schematic - ASTID Project



1. A.M. Skelley et al., *SPIE: Proceedings of the In-Situ Instrument Technologies Meeting*, 4878 (2002) 59.
2. E.T. Lagally et al., *Lab-on-a-Chip*, 1 (2001) 107. Integrated PCR with CE analysis of nucleic acids
3. E.T. Lagally et al., *Anal. Chem*, submitted (2004). Portable bacterial detection and typing instrument

Berkeley, UCSD and JPL

Mars Organic Analyzer (MOA)

Acknowledgements

- Alison Skelley, Jim Scherer and Will Grover

- ASTEP - *Microfabricated Organic Analyzer (MOA) for in situ Exploration of Mars and Other Solar Bodies* PI's Mathies, Grunthaner and Bada, 1/04 -1/07.

- ASTID - *Integration of a Micro-chip Amino Acid Chirality Detector into the Mars Organic Detector,* subcontract to UCB from UCSD, PI Bada, 5/02 - 5/04.

- JPL Instrument Development Funds

- NSERC Fellowship to Alison Skelley

- UC Berkeley Microfabrication Laboratory