



Detection of Trace Biomarkers in the Atacama Desert with the UREY *in situ* Organic Compound Analysis System

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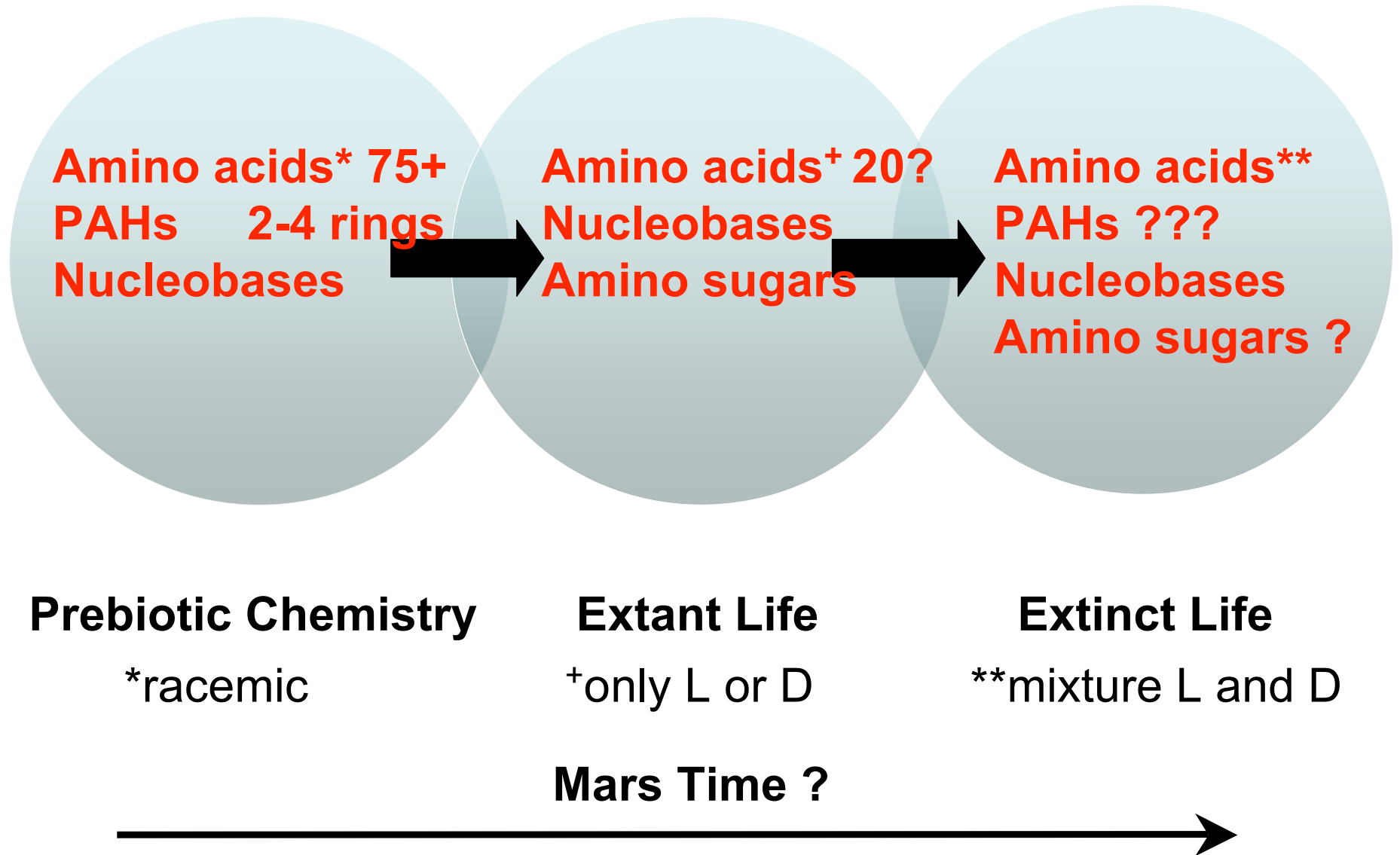
Scripps Institution of Oceanography

Jet Propulsion Lab - Cal Tech

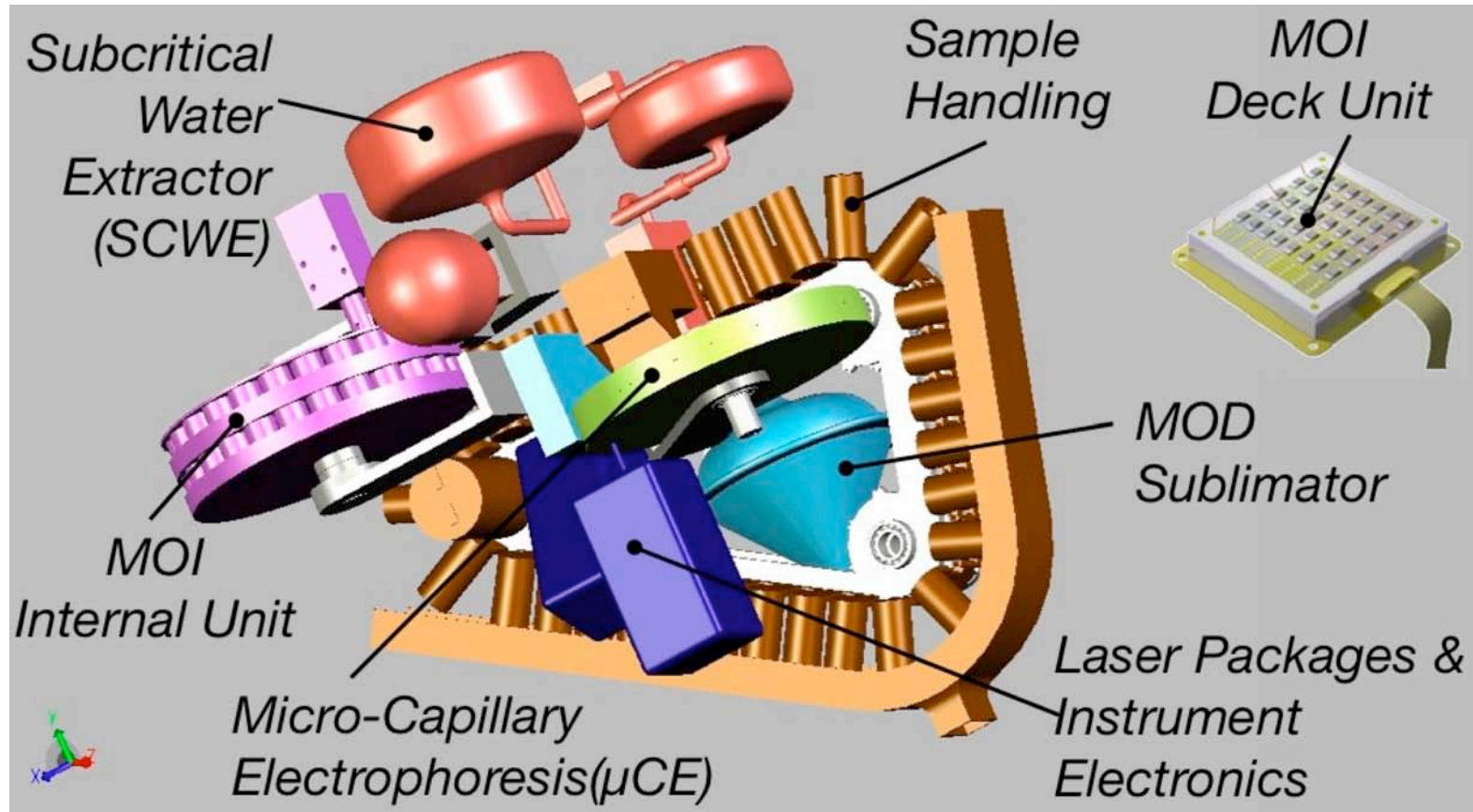
University of Leiden

NASA Ames

Molecular Signatures of Planetary Evolution



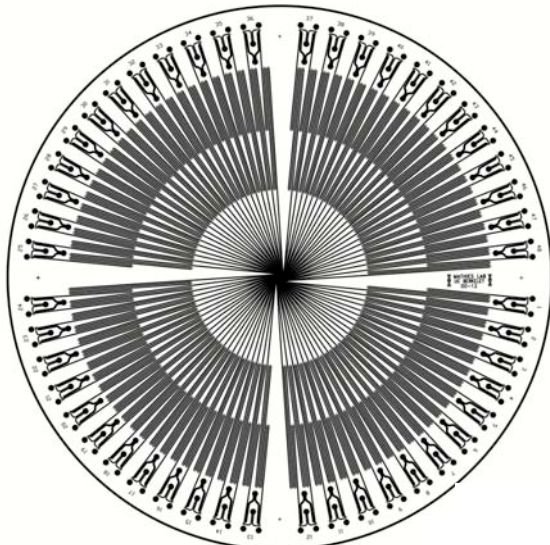
UREY Instrument Suite for Biomarker Detection



- Sublimation and sub-critical water extraction of bioorganics from soil
- Composition analysis of extracted amino acids, amines, and nucleobases
- Chirality analysis of amino acids to determine biological origin
- Urey has been selected by ESA for the Pasteur ExoMars 2011 Mission

Microfabrication Technical Heritage

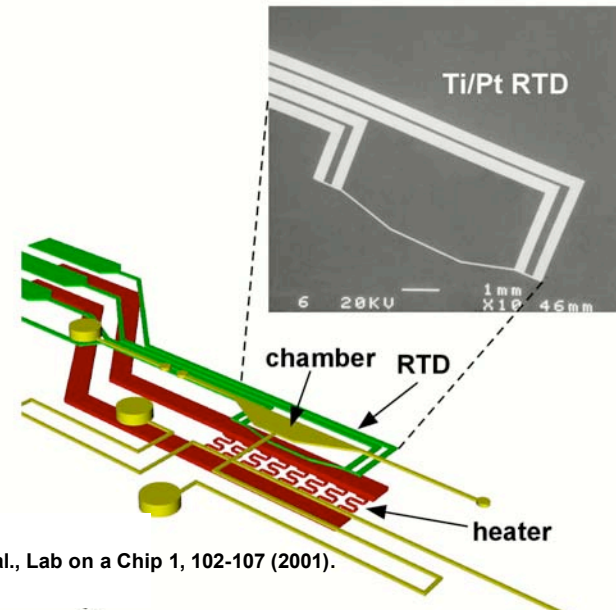
Capillary Array Sequencing Bioprocessor



Paegel et al., Proc. Natl. Acad. Sci. 99, 574-579 (2002)

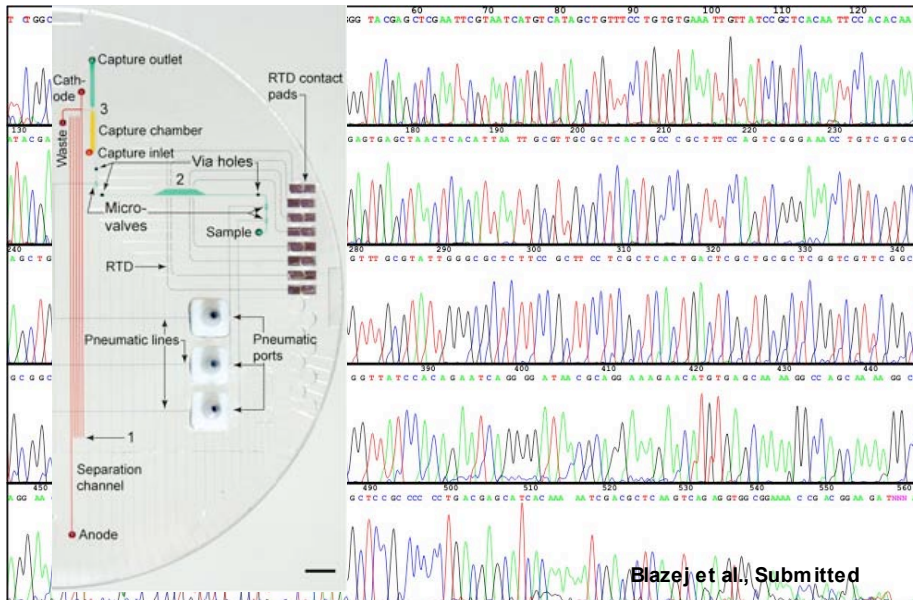
- Integration of 384 electrophoresis channels for parallel analysis
- and clean-up reactors for DNA sequencing

Integrated PCR-CE Microdevice



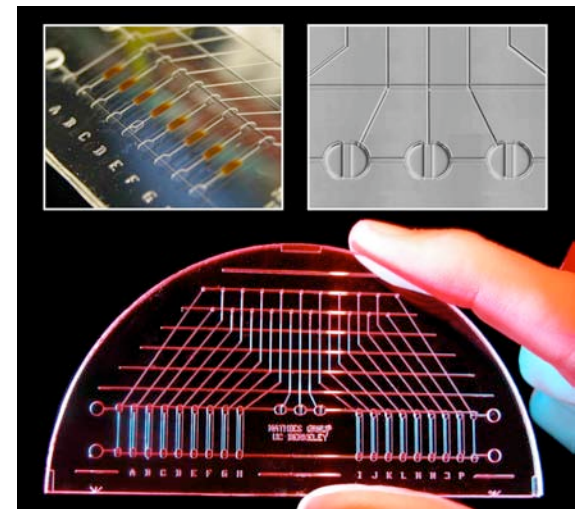
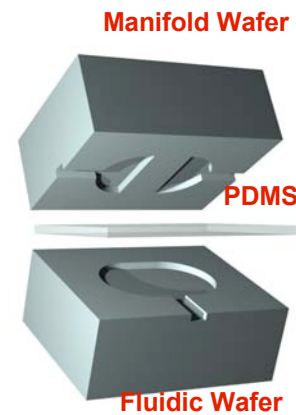
Lagally et al., Lab on a Chip 1, 102-107 (2001).

Integrated Thermal Cycling Capture and Sequencing



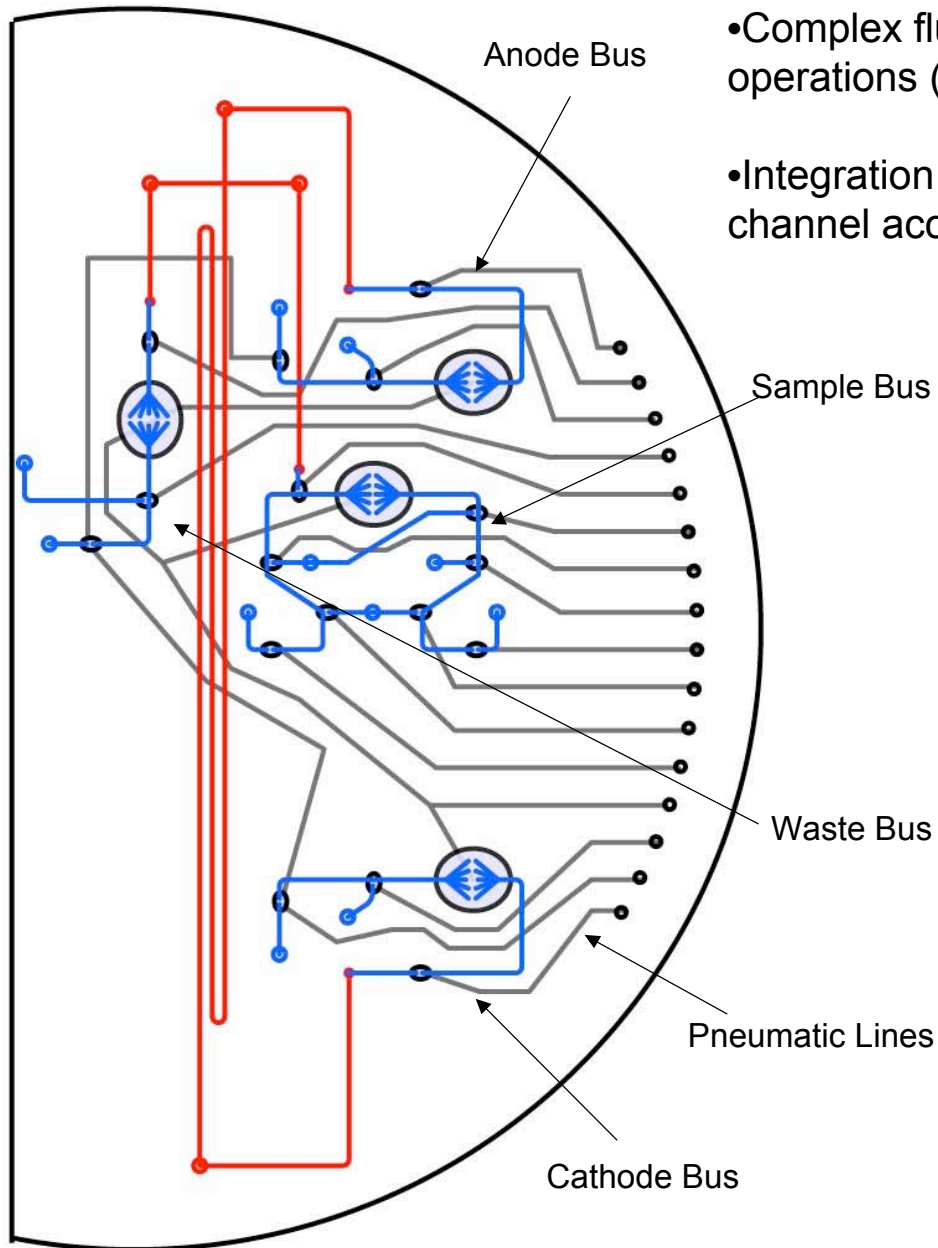
Blazej et al., Submitted

Integrated PDMS Valves and Pumps for Molecular Computation

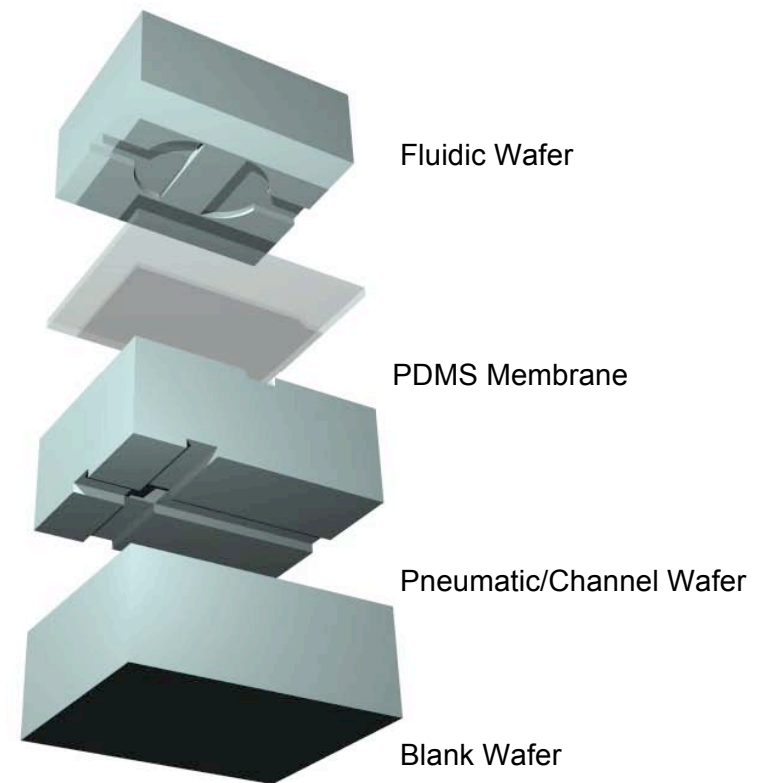


Grover et al., Sens. Actuators B. 3, 315-323 (2003).
Grover et al., Lab on a Chip 5, 1033-1040 (2005).

Microfabricated Device for Automated Analysis

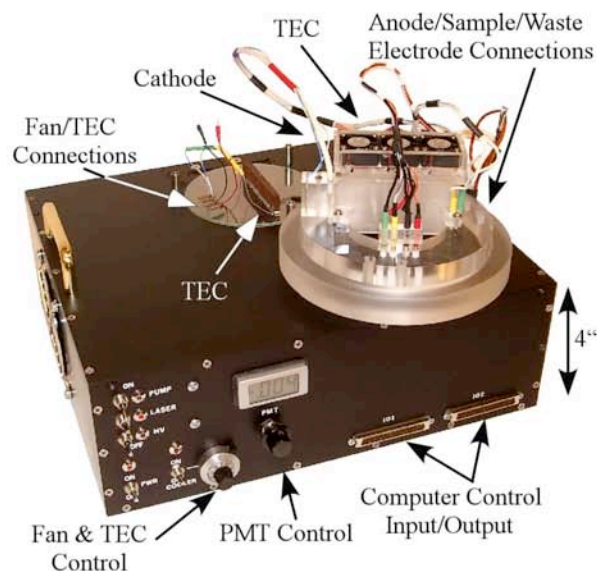


- Complex fluidic bus structures allow precise fluidic operations (loading, rinsing, diluting etc.)
- Integration of PDMS fluidic bus structures and CE channel accomplished with a 4-layer device



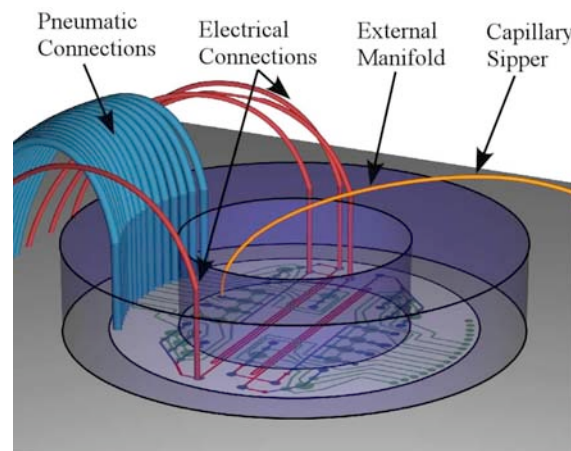
The Mars Organic Analyzer

Exterior of the Portable Instrument



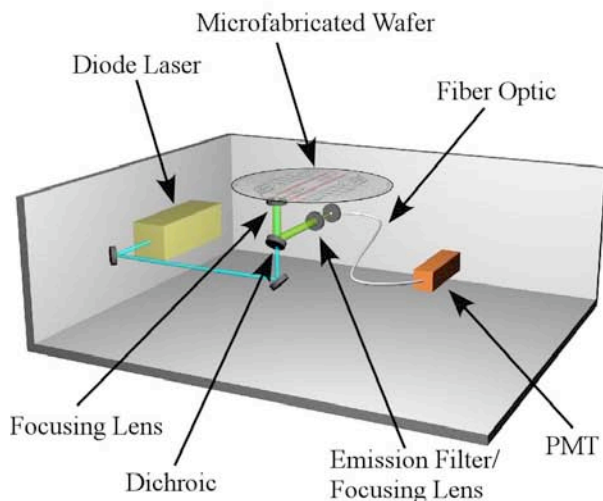
- Portable instrument includes electronics, pneumatics, CE power supplies, optics
- ~ 11 kg, peak power consumption ~ 15 W

External Manifold



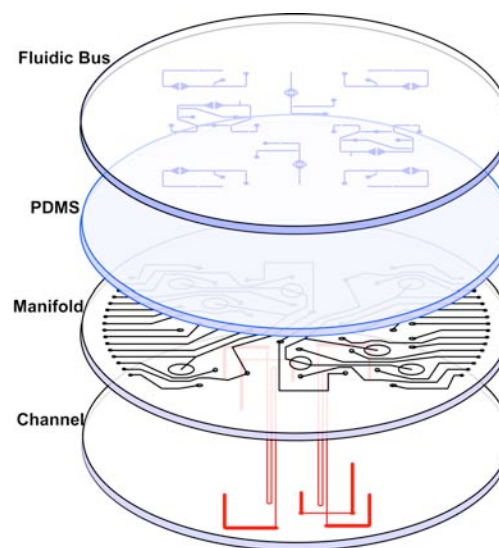
- External manifold interfaces with microfabricated device
- 4 electrical and 16 pneumatic connections
- Capillary sippers bring in samples and buffers

Interior of the Portable Instrument



- Confocal optics and custom-built objective (NA ~ 0.9) yields high sensitivity detection

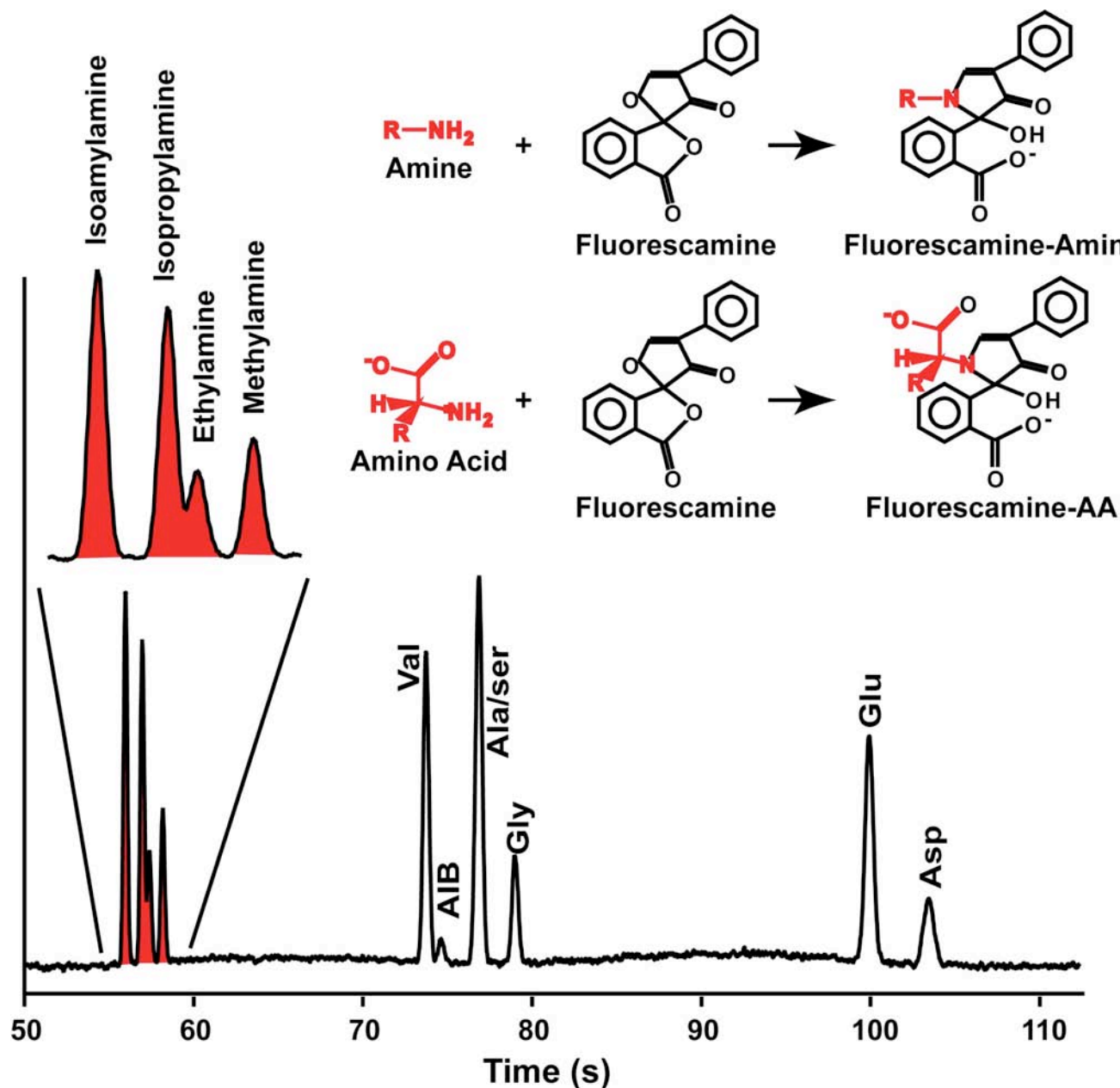
4-Layer Microfabricated Device



- 4-layer device integrates CE channels and PDMS valves and pumps
- Complex fluidic routers enable autonomous operation

A.M. Skelley *et al.* (2005) *PNAS* **102**, 1041-1046.

Analysis of Amino Acids and Amines

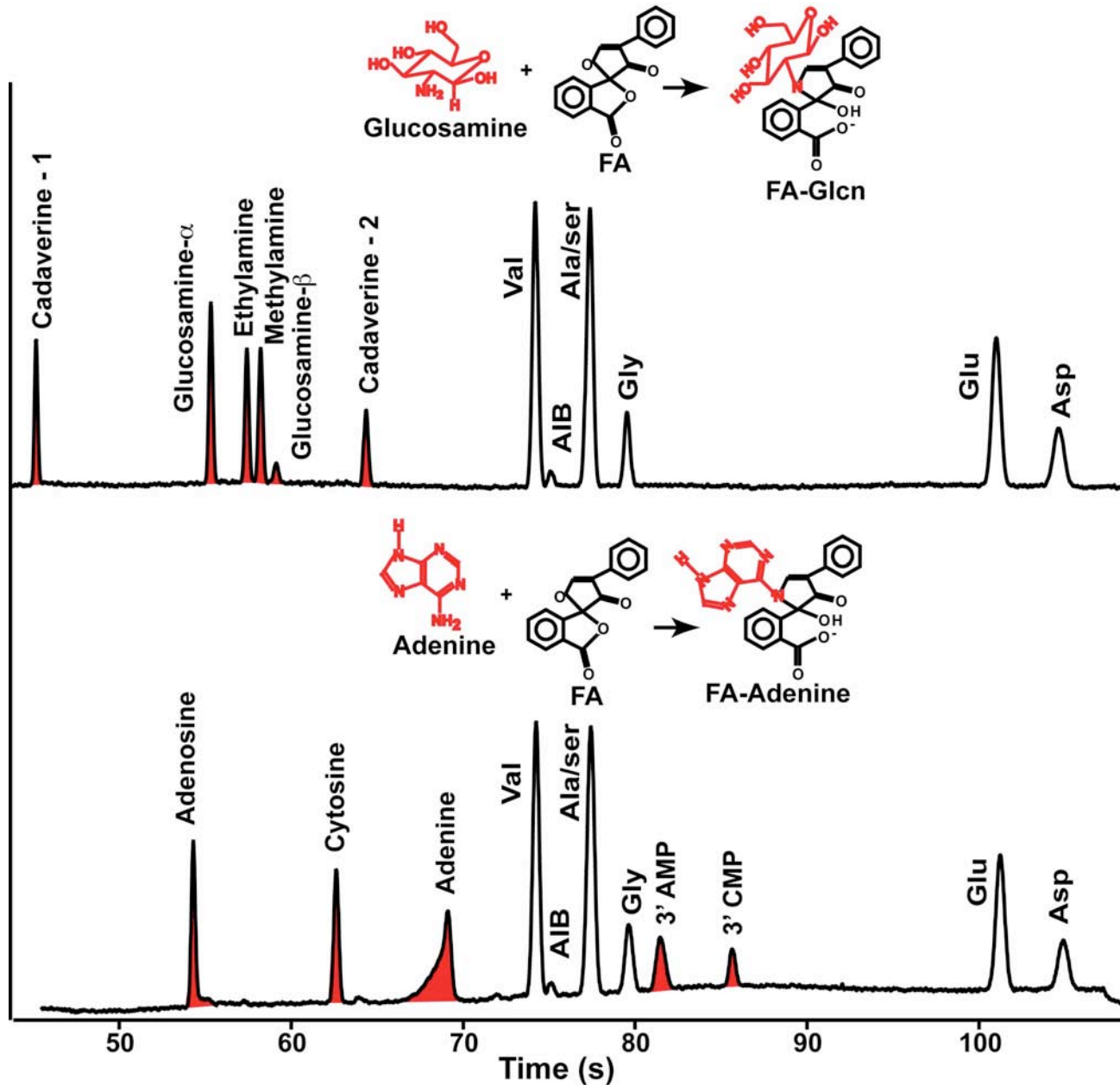


- Amines and amino acids are important tracers of biological activity

- Small amines (methyl and ethylamine) are biodegradation products

- High resolution separations (one methyl mass difference) can be achieved

Analysis of Diamines, Amino Sugars, and Nucleobases



- Diamines are biodegradation products

- Glucosamine is found in the shells of arthropods

- Both singly and doubly labeled diamines and both glucosamine anomers are resolved

- Nucleobases and their derivatives are easily labeled and detected

Validation of the MOA for Astrobiology Exploration

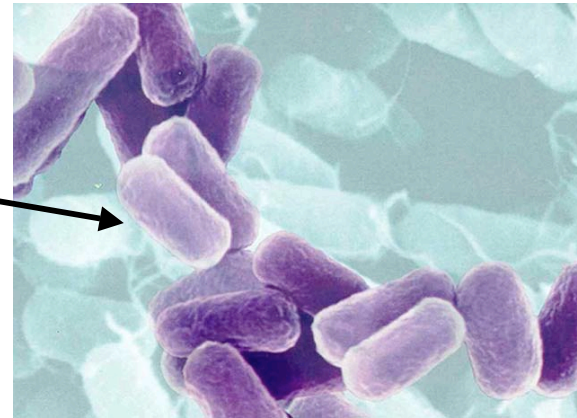
- **Demonstration of ppb to ppt amine and amino acid detection sensitivity**

- **Analysis of amines and amino acids from Jarosite: a mineral found by MER on Mars**



- **Detection of amines from low levels of bacteria in soil**

- **Analysis of amines, amino acids and nucleobases from prebiotic synthesis**



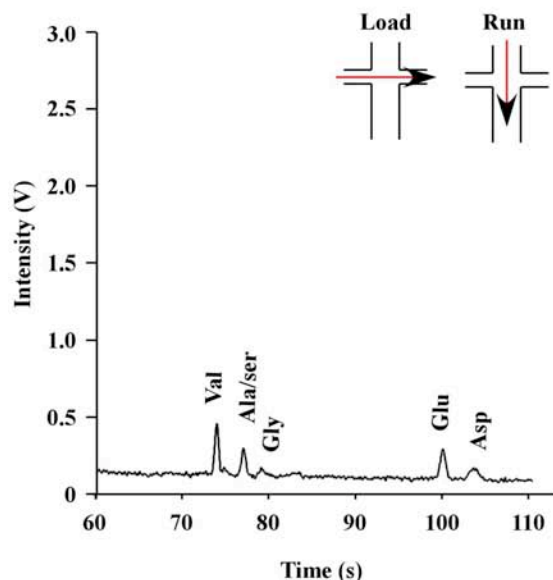
- **Demonstration of automated multisample operation**

- **Field tested in the most relevant terrestrial location:
The Atacama Desert, Chile**

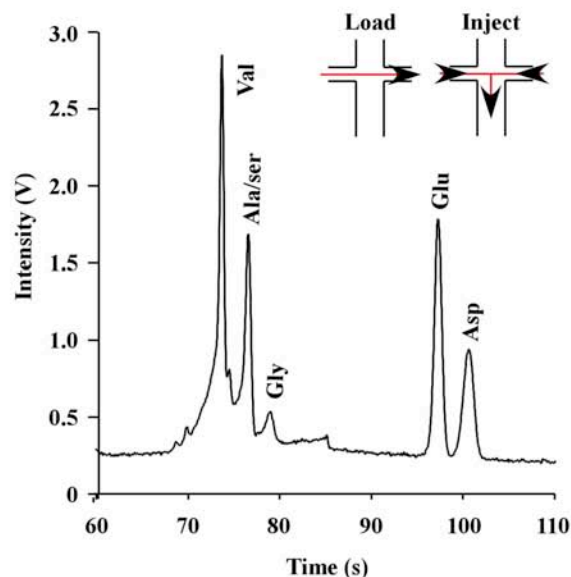


Determination of MOA Sensitivity

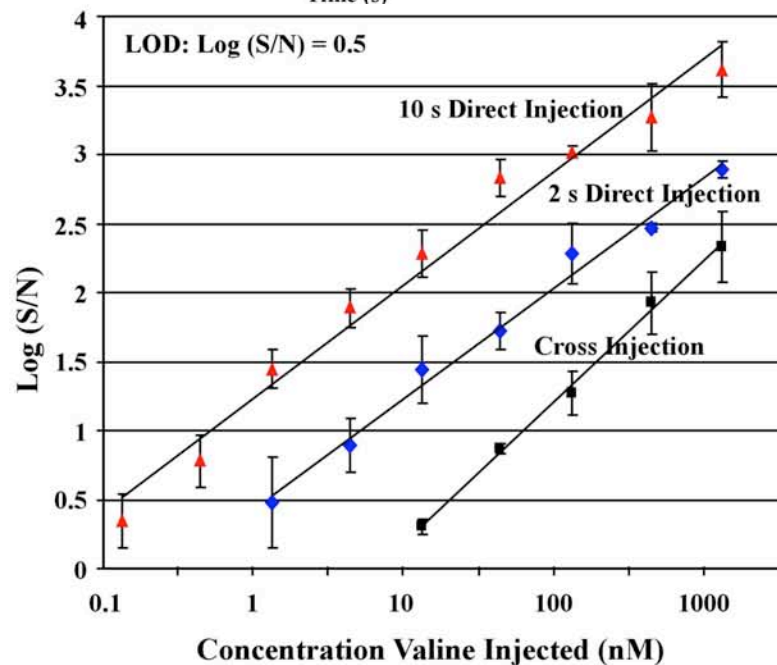
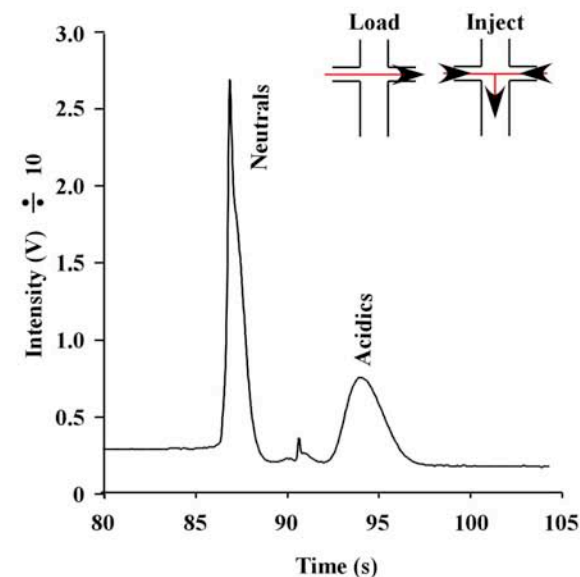
Cross Injection



2 s Direct Injection



10 s Direct Injection



Injection Style

Cross Injection

2 s Direct Injection

10 s Direct Injection

LOD

13.3 nM

1.33 nM

133 pM

pptr (1 g soil)

500

50

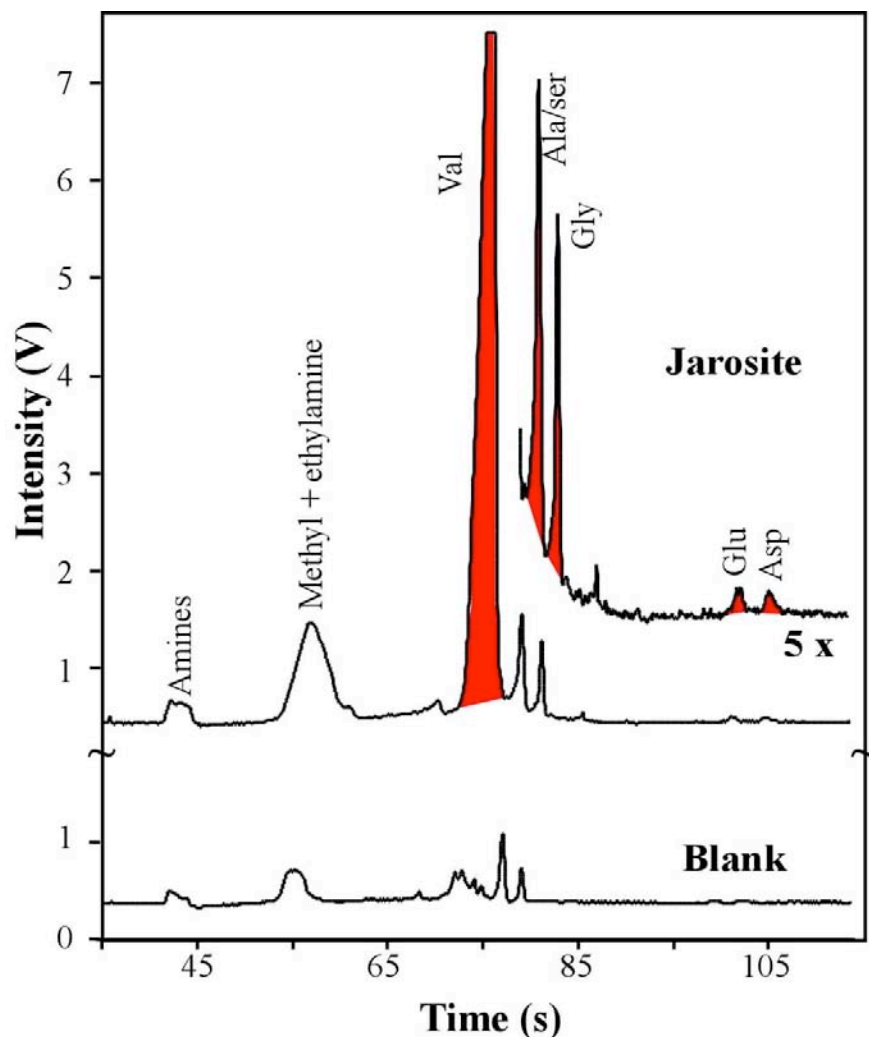
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• Microfabricated CE analyzer has demonstrated pM and parts-per-trillion sensitivity

A.M. Skelley *et al.* (2005) *PNAS* **102**, 1041-1046.

Analysis of Amines and Amino Acids from Jarosite

MOA analysis of MOD sublimation samples



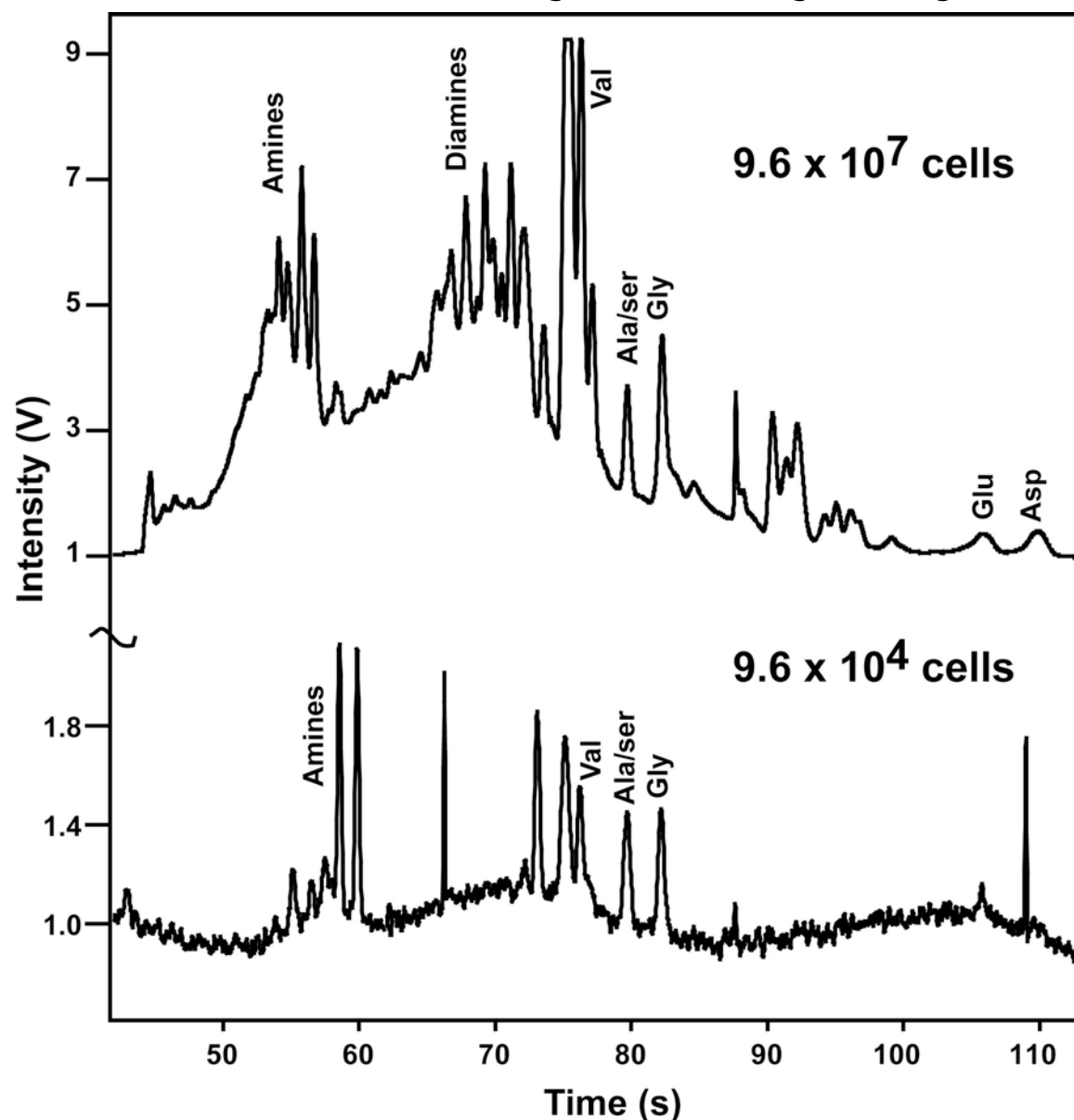
MOA and HPLC analysis of jarosite samples

Sample	Amino Acid	Concentration (ppb)	
		HPLC	CE
Field	Val	not run	$\sim 100 \pm 30$
Sublimed	Ala/Ser	"	0.4 ± 0.2
Solid Vein	Gly	"	0.20 ± 0.09
	Glu	"	0.07 ± 0.03
	Asp	"	0.13 ± 0.08
Lab	Val	60 ± 30	94 ± 9
Extracted	Ala/Ser	170 ± 40	160 ± 10
Solid Vein	Gly	121 ± 10	12 ± 2
	Glu	40 ± 40	79 ± 5
	Asp	60 ± 20	65 ± 5

- The MOD-MOA combination successfully extracted and analyzed amines and amino acids from jarosite
- Amines and amino acids are preserved in acidic jarosite soils

A.M. Skelley *et al.* (2005) *PNAS* 102, 1041-1046.

MOA Analysis of Hydrolyzed *E. Coli* Cells

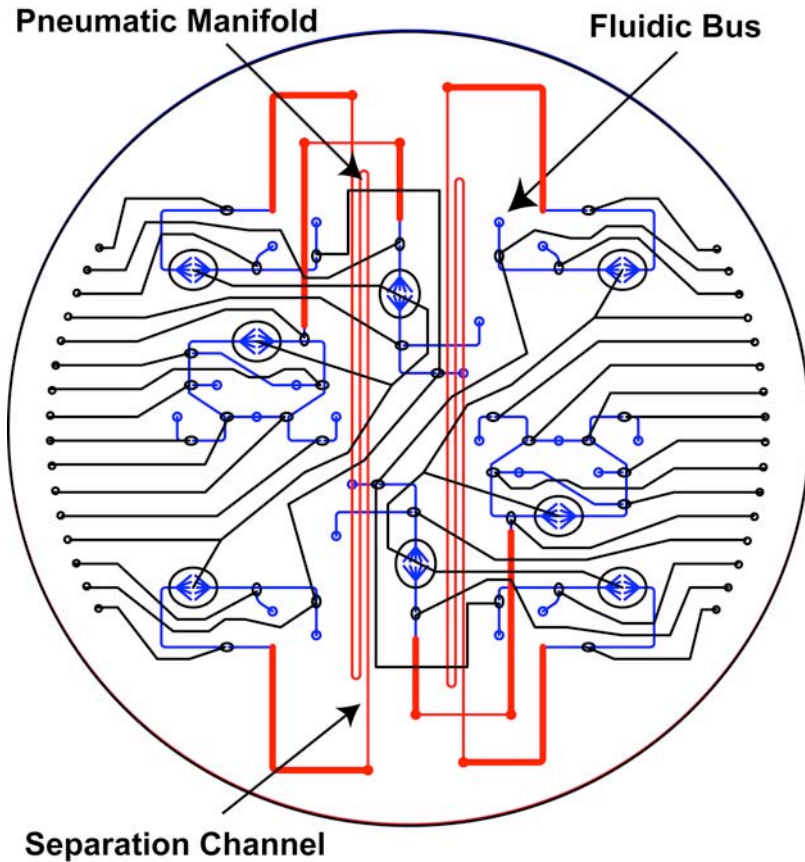


- Bioamines from 10^8 to 10^5 cells were extracted and labeled with fluorescamine
- Linear response over 4 orders of magnitude was found
- At 10^5 cells, amines and amino acids were still easily detectable with a cross injection
- Limits of detection: $\sim 10^4$ cells for cross injection, $\sim 5 \times 10^3$ cells for 300 ms direct injection

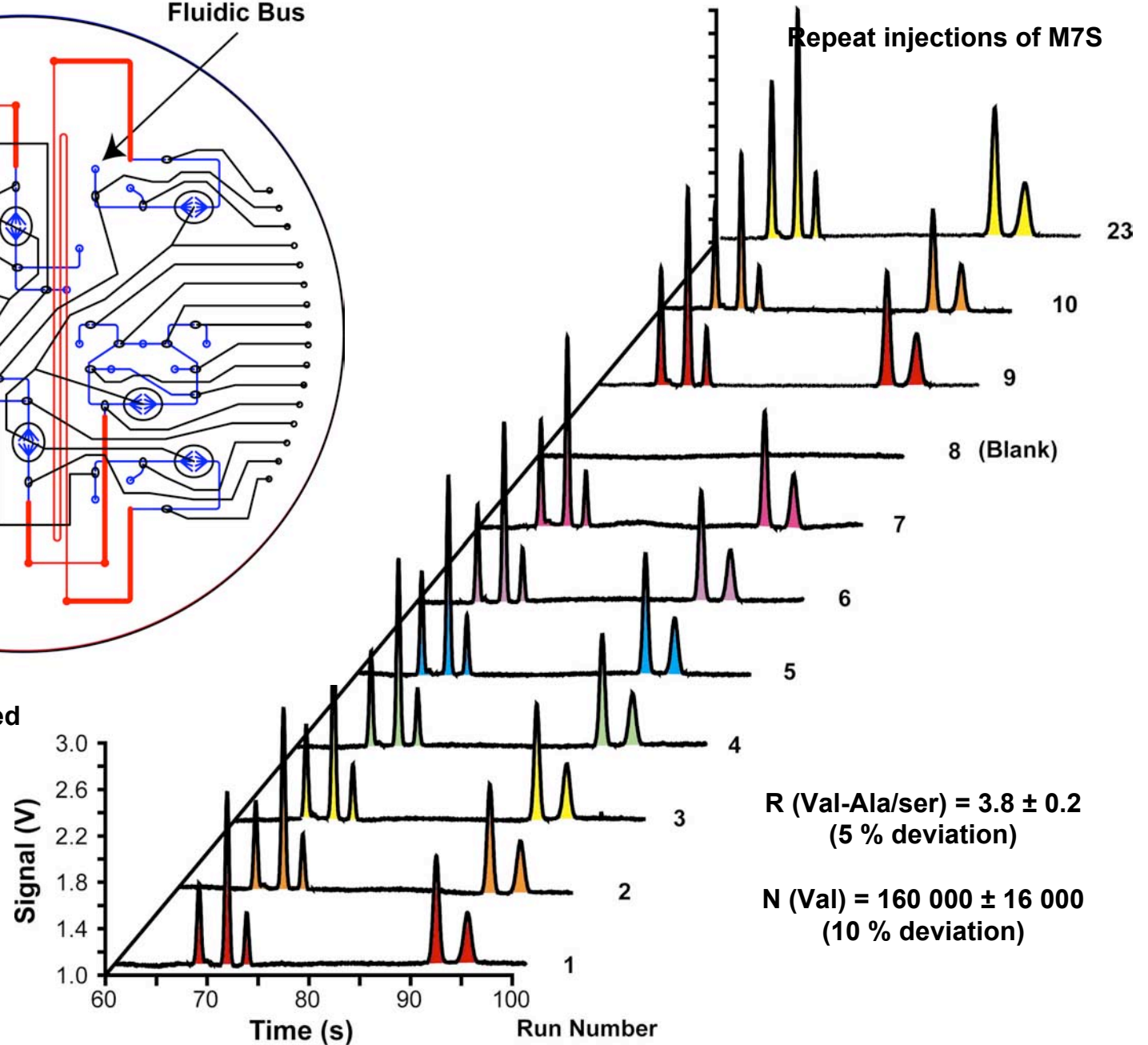
The MOA can detect bioamines from $\sim 5 \times 10^3$ hydrolyzed *E. coli* cells

A.M. Skelley *et al.* Manuscript in Preparation.

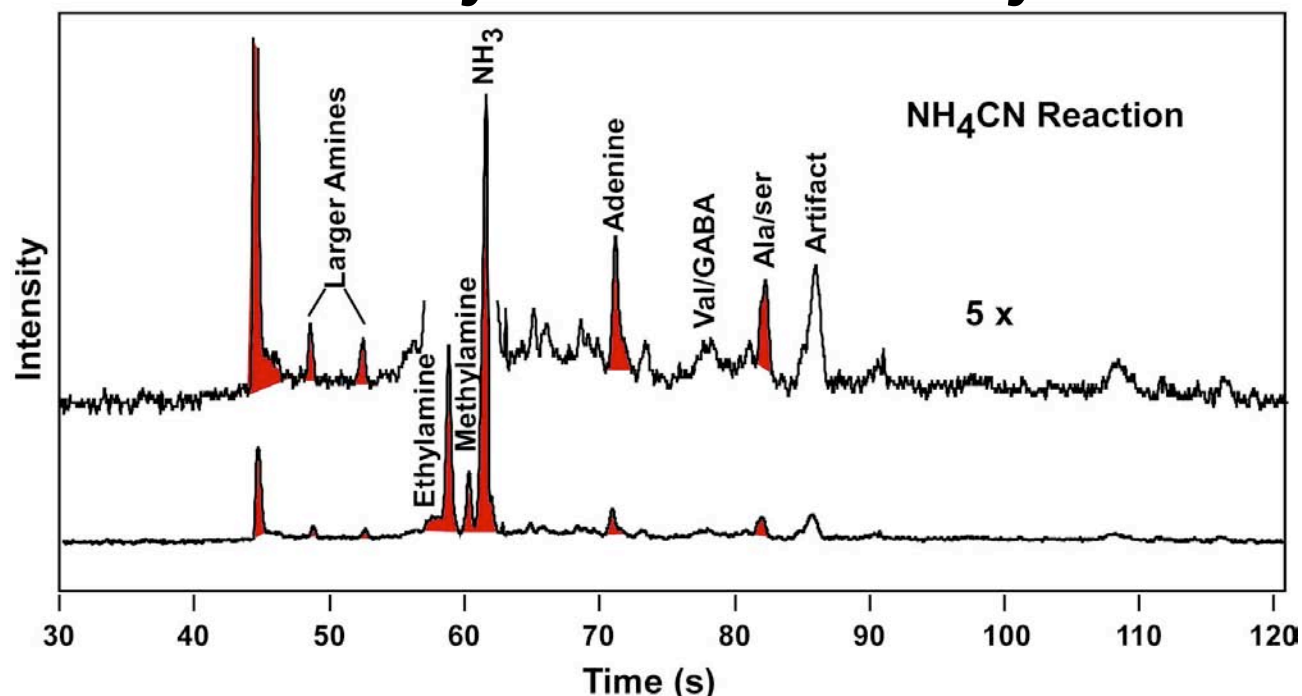
Automated Analyses of Multiple Samples using the MOA



- The device was programmed with off-chip reservoirs of buffer, sample and waste connected via sippers
- EOF used to clean between runs



Analysis of Prebiotic Synthesis Products



- 1 M NH₄CN was reacted at 100 °C for 5 days

- Products were extracted using sublimation but no sample cleanup was performed

- Adenine and alanine/serine were detected at μM and nM concentrations (ppm to ppb concentrations in a 0.2 g soil sample)

- Percentage yield was determined based on moles of product versus moles of starting reagent (NH₄CN)

- Adenine was produced at $0.04 \pm 0.0004 \%$, while alanine/serine was produced at $0.00035 \pm 0.0001 \%$

Key biological molecules are detected from a representative prebiotic synthesis with only a sublimation extraction

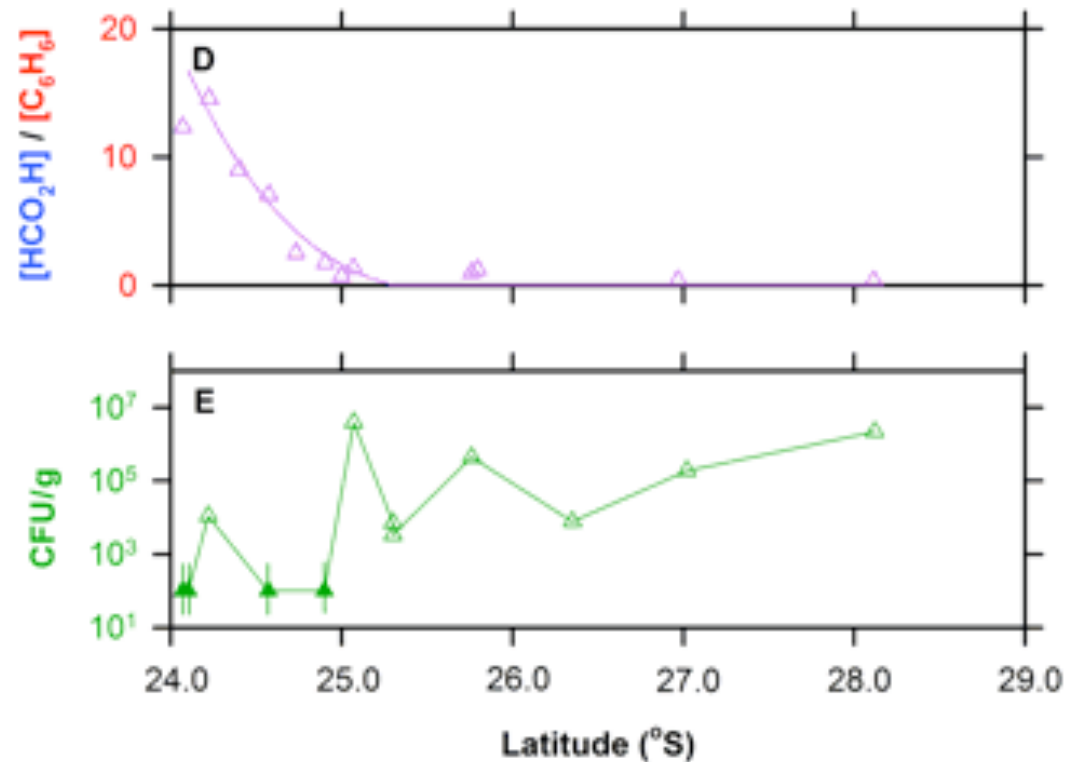
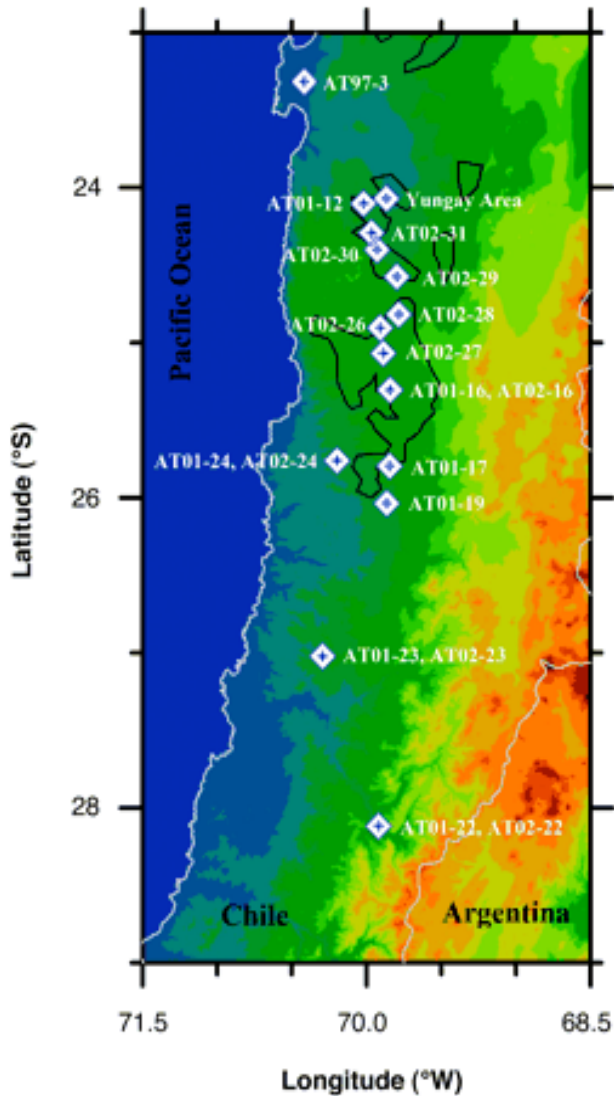
The Atacama Desert, Chile

- Extremely low levels of rainfall (<1 mm/yr)
- No overt signs of indigenous life in the Yungay region
- Highly oxidizing soils
- An excellent location to test *in situ* Mars analyzers for extraterrestrial life



North-South Transect of the Atacama

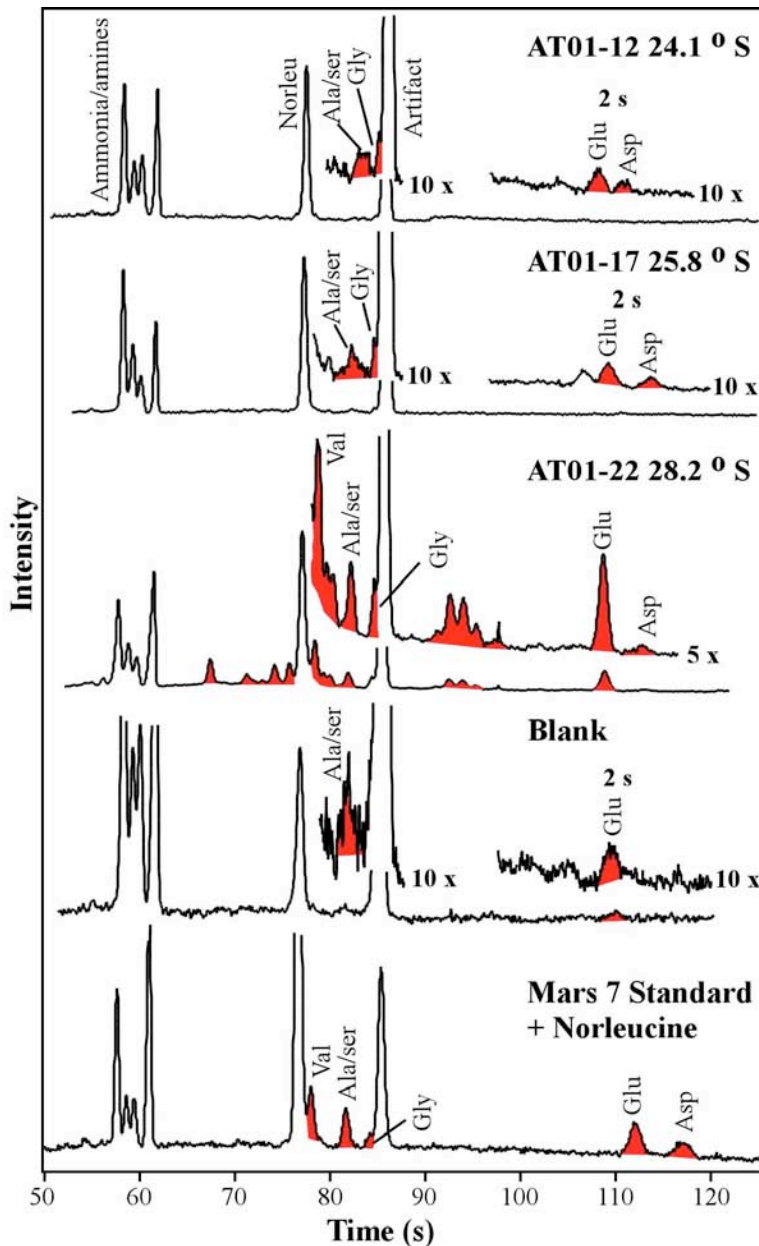
North-south transect of the Atacama Desert



- The Yungay region is the arid core of the Atacama
- Extremely low levels of culturable bacteria, highly oxidized organics (only benzene and formic acid detected)

Lab-Based Analysis of Atacama Samples using MOA

MOA analysis of Atacama extracts



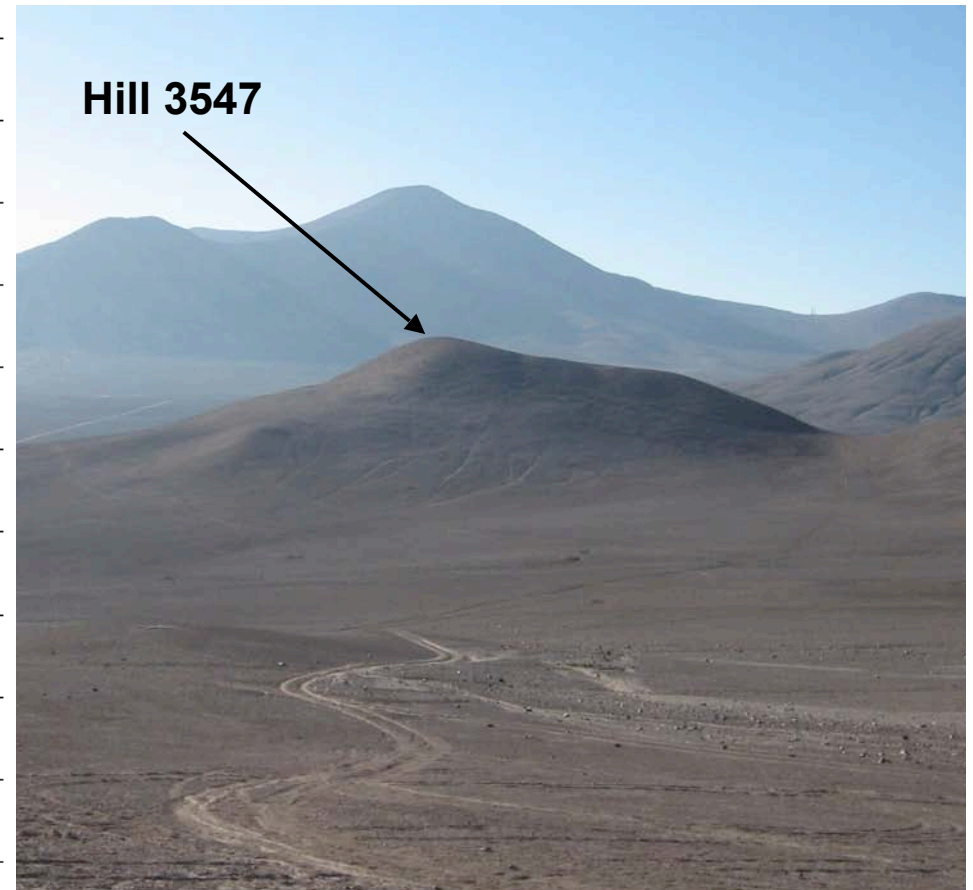
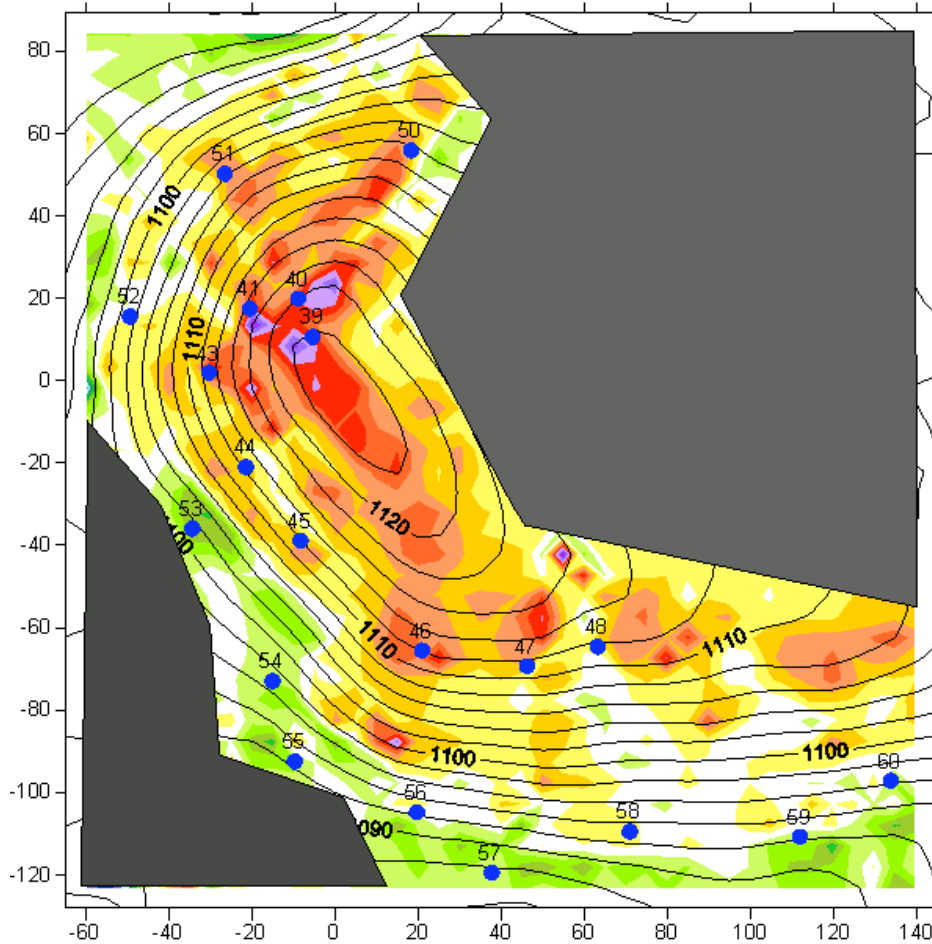
MOA and HPLC analysis of Atacama extracts

Sample	Amino Acid	Concentration (ppb)	
		HPLC	CE
AT01-12 24.1° S	Ala/Ser	31 ± 3	9 ± 6
	Gly	bb	30 ± 30
	Glu	52 ± 5	bb
	Asp	bb	10 ± 0.01
AT01-17 25.8° S	Ala/Ser	27 ± 3	10 ± 20
	Gly	bb	20 ± 40
	Glu	72 ± 7	bb
	Asp	bb	10 ± 10
AT01-22 28.2° S	Val/γ-ABA	390 ± 40	560 ± 40
	Ala/Ser	510 ± 50	170 ± 20
	Gly	73 ± 7	320 ± 70
	Glu	420 ± 40	360 ± 70
	Asp	140 ± 10	40 ± 10

•The MOA successfully analyzed amino acids from soil samples at the dry limit of microbial life on Earth

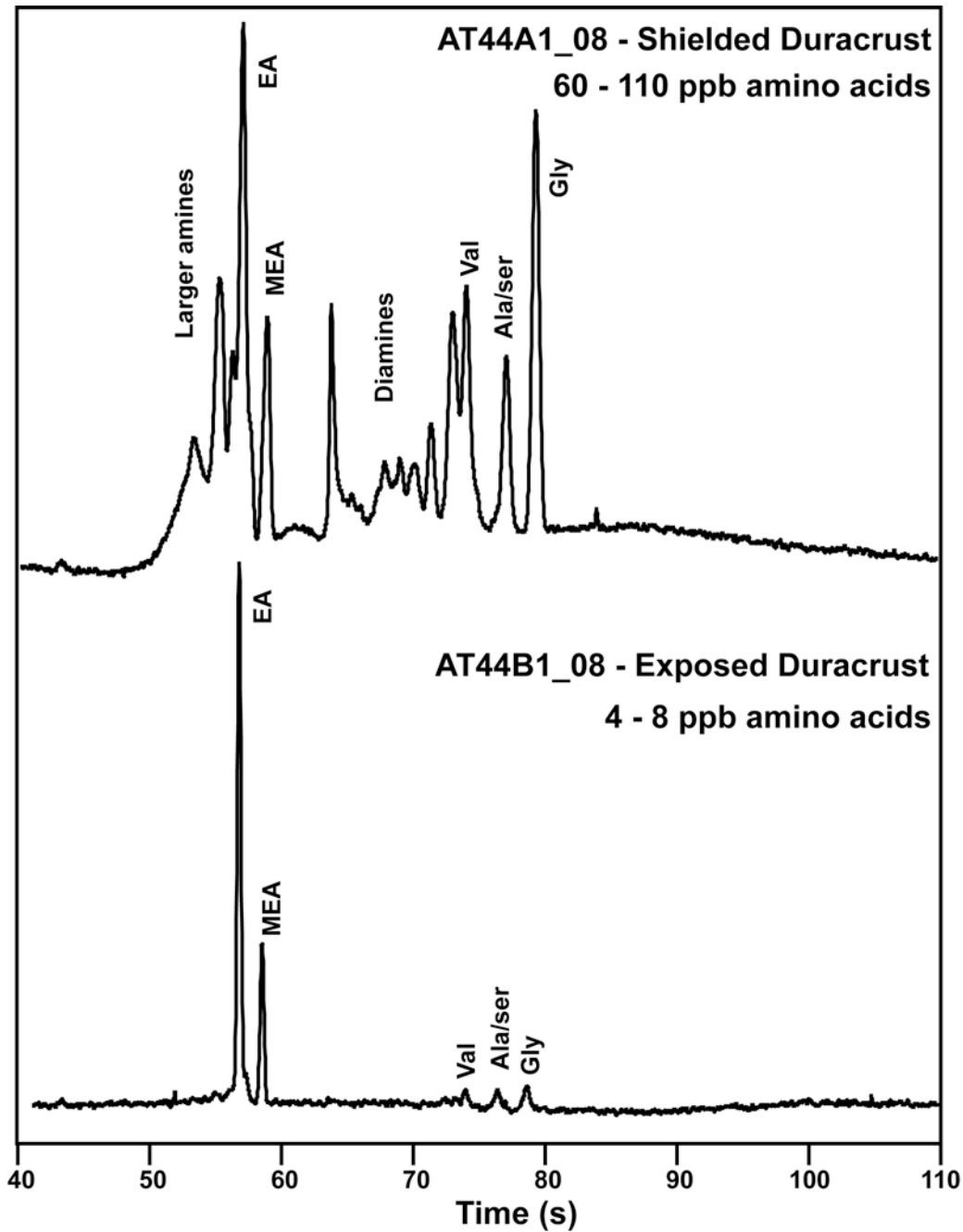
A.M. Skelley *et al.* (2005) *PNAS* **102**, 1041-1046.

Hill 3547 Sample Site, Atacama Desert, Chile



- Located in the Yungay Region, adjacent to the Rock Garden
- Detailed sampling performed at all sites
- Samples processed by Sub Critical Water Extractor (SCWE) then analyzed by MOA
- **340 separations** performed on **only 3 channels** over **2 week period**
- Instruments operated at all temperatures (~ 3 C to ~ 30 C).

Site 44: Shielded vs. Exposed Duracrust Microenvironments



Shielded Duracrust



Exposed Duracrust

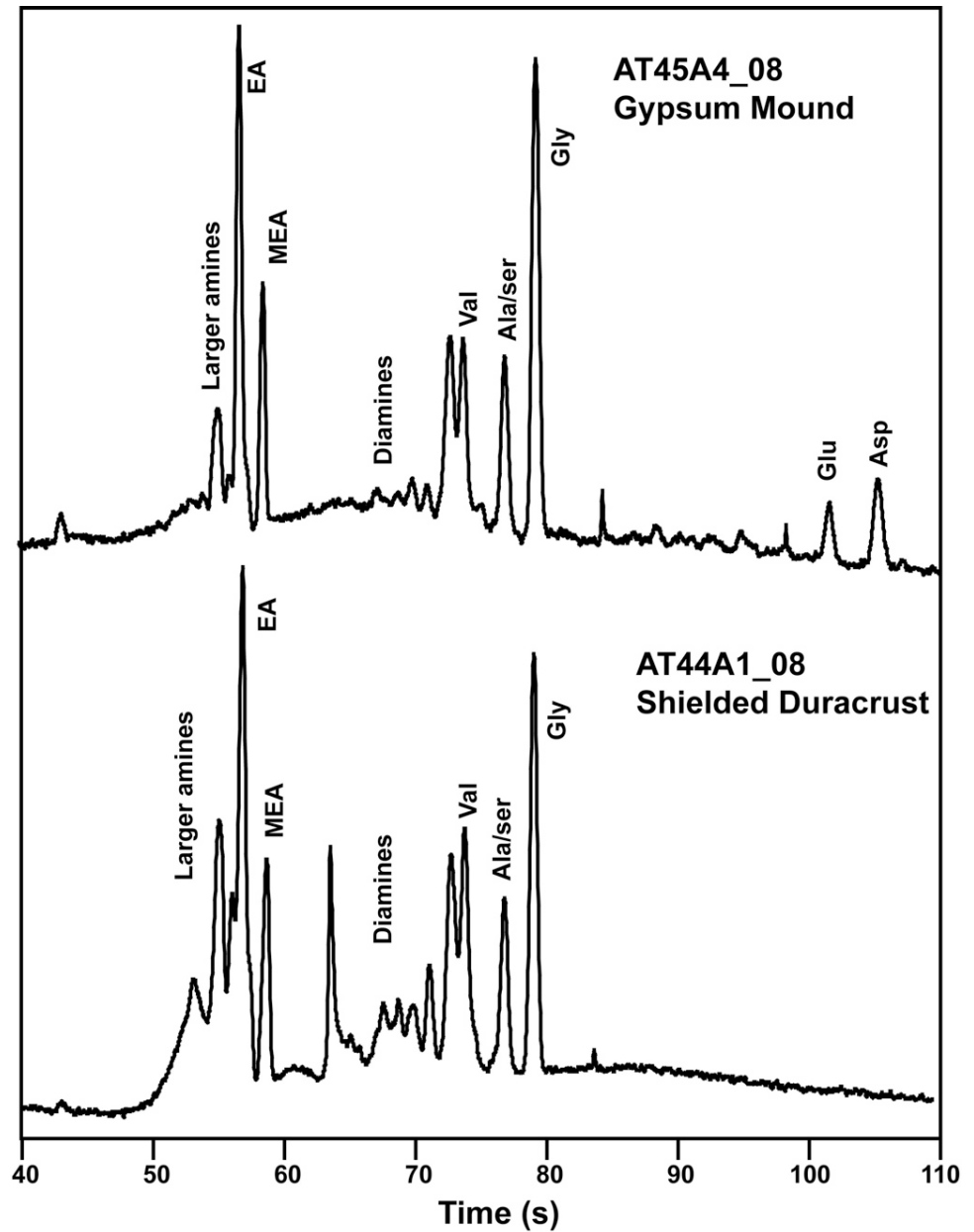
Site 45: Gypsum Mound Above Sign of Past Water Flow



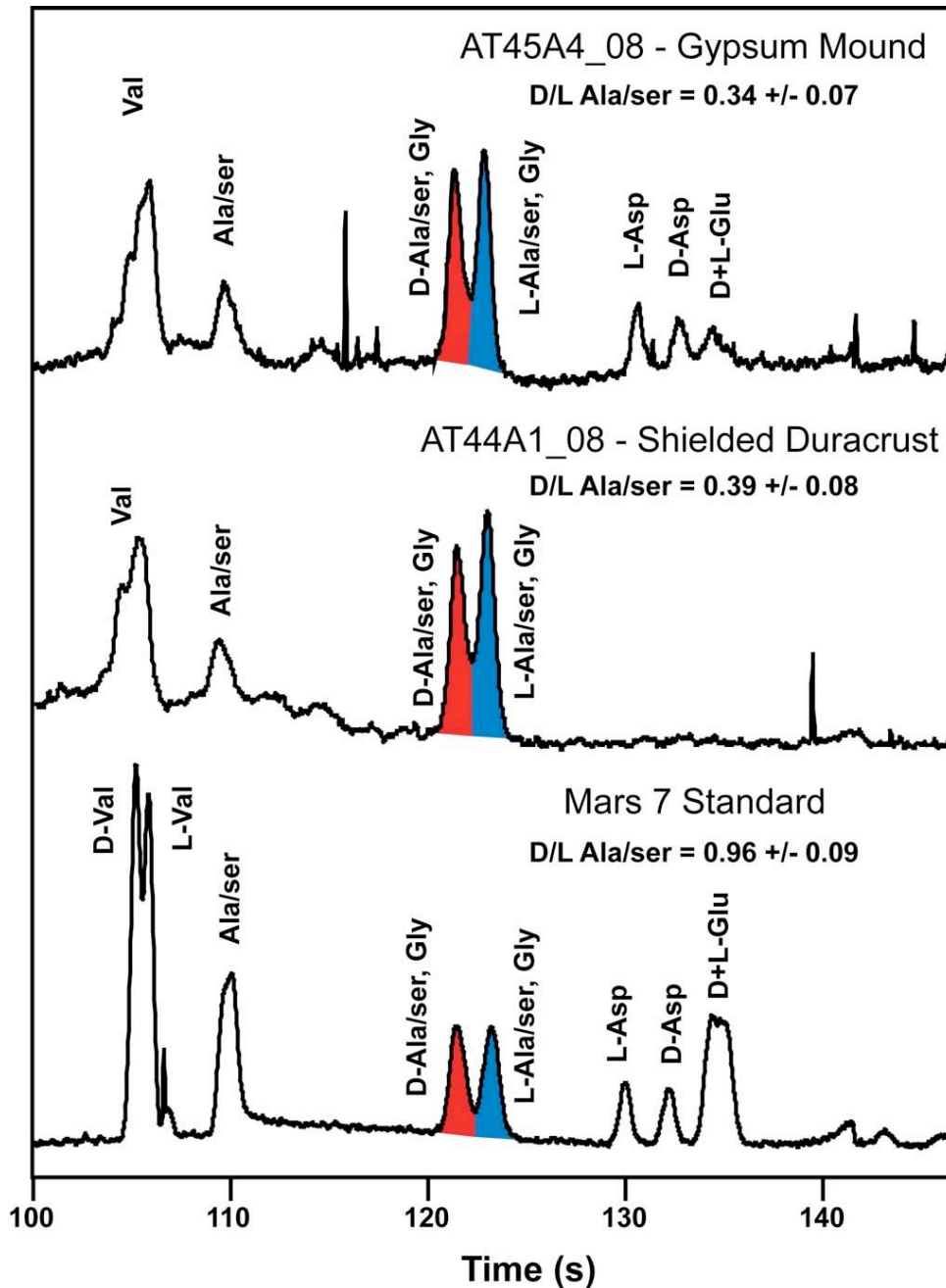
Gypsum mound at site 45



Flow at site 54



Preliminary Chiral Analysis of Atacama Samples



Composition Analysis

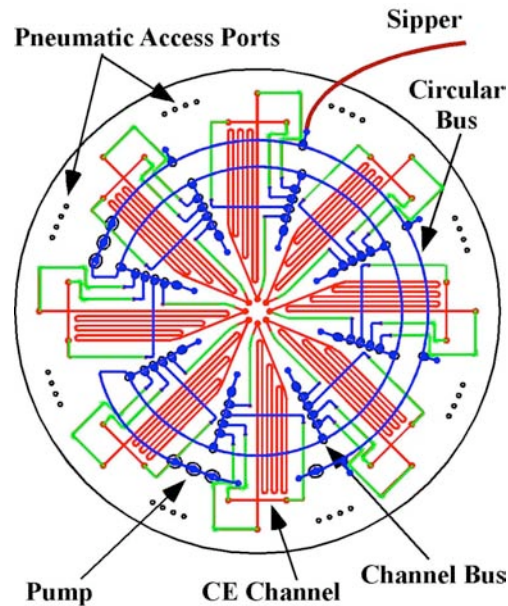
- We see $\sim 4.7 \times 10^{-16}$ mol valine per E. coli cell in our experiments
- Observed valine signal corresponds to about 1×10^6 cells/g at Site 44 and 2×10^5 cells/g at Site 45
- 1×10^2 to 3×10^5 CFU/g were reported by Navarro-Gonzales

Chirality Analysis

- At the average temperature in the Atacama (~ 16 °C), the racemization rates are 7×10^{-6} yr⁻¹ for dry conditions and 5×10^{-4} yr⁻¹ for wet conditions
- Based on the observed D/L ratios the calculated sample ages are:
 - 8×10^3 to 6×10^5 yr for asp
 - 2×10^3 to 1×10^5 yr for ala/ser

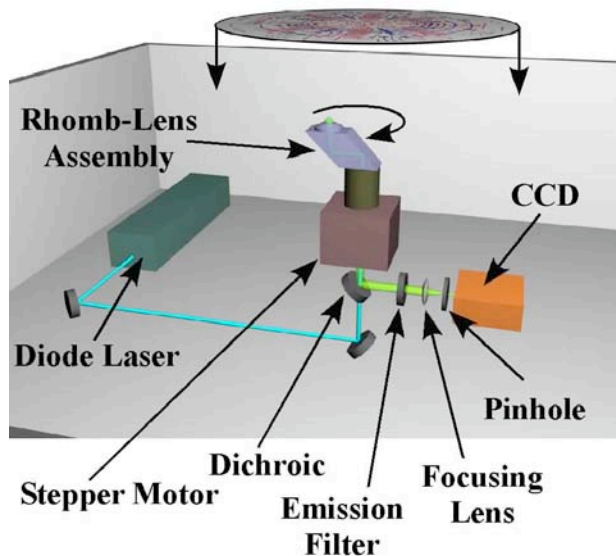
Development of an Automated, Multi-Channel Instrument

Microfabricated Device



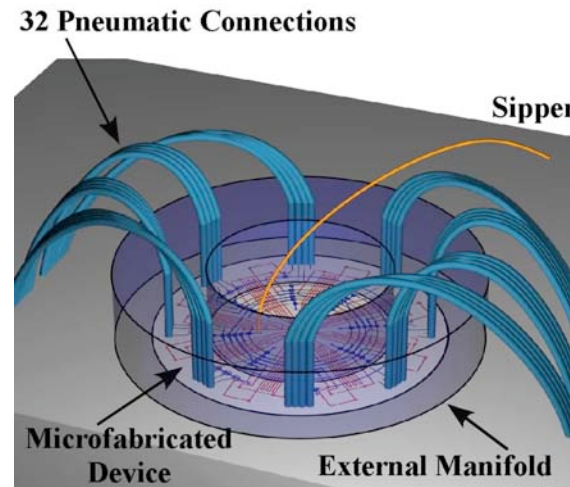
- 8 separation channels
- 2-stage bus
- 32 pneumatic lines

Confocal Scanning Optics

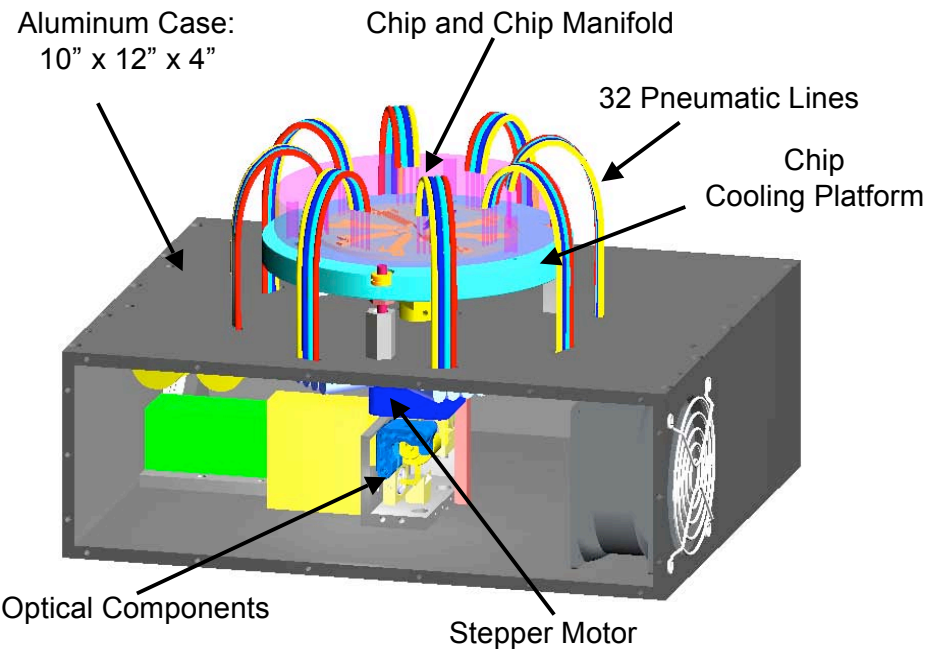


- Device is fixed prior to flight
- Scanning objective locates channel for analysis

External Manifold



- External manifold interfaces with microfabricated device
- 4 switchable power supplies





Acknowledgements

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For more info:

<http://astrobiology.berkeley.edu>

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