Nanofluidic Size Exclusion Chromatograph for In Situ Macromolecualr Analyses

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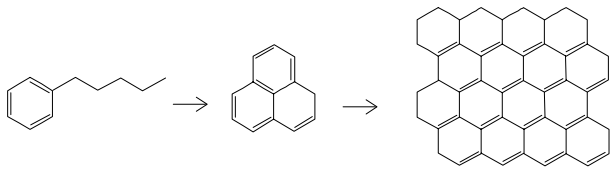
Overview

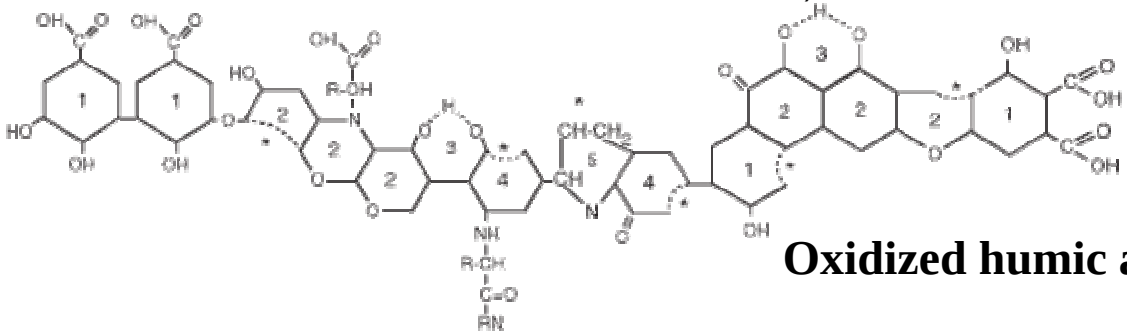
- Size exclusion chromatography (SEC) is a subset of high performance liquid chromatography in which high molecular weight species are separated based on their retention time in a size exclusion column.

- We have begun development of a novel nanofluidic SEC-on-a-chip consisting of a nanofabricated size exclusion column with on-chip laser-induced fluorescence detection (eventually switch to nano-ESI & TOF-MS)

- The proposed instrument relies on a simple, universal separation mechanism based on molecule size and is very well suited for separation and analysis of complex mixtures in an unknown chemical state using both aqueous and organic solvents

Organic molecule alteration





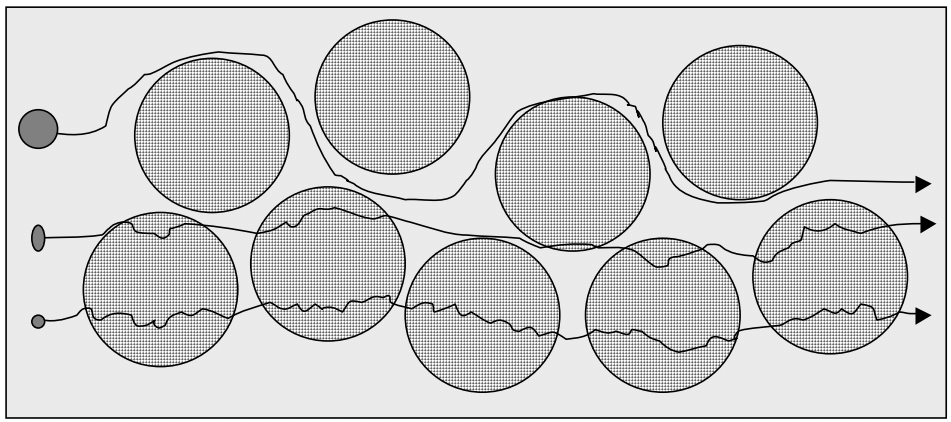
Oxidized humic acid molecule

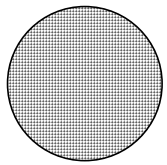
Becker *et al*: Macromolecular kerogen-like compounds present in Martian meteorites
McDonald: Humic substances may be stable over billions of years under plausible Martian surface conditions

<u>Size Exclusion Chromatography is a standard technique on Earth for studying humic substances, oils, bitumen, and other organic macromolecules found in soil & sediment samples</u>

Size Exclusion Chromatography: Principles of operation

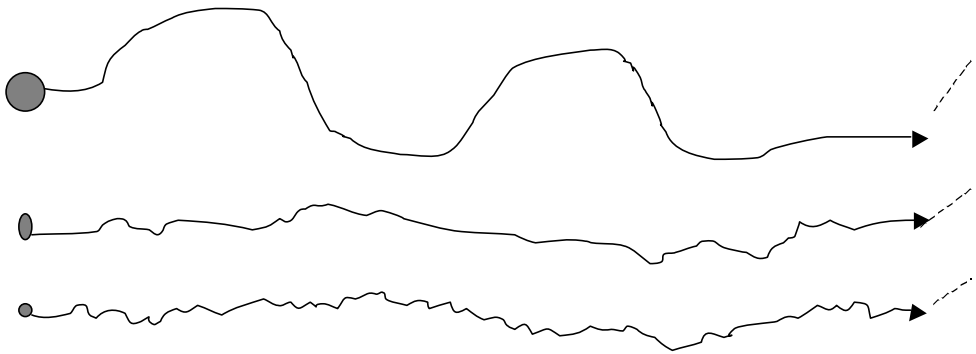
In conventional size exclusion chromatography, columns are packed with silica or polymer beads containing a network of uniform nanopores. Molecules will follow different paths through the size exclusion network based on their molecular diameter, with characteristic elution times that can be related to molecule size.





Silica or polymer bead

The conventional size exclusion separation column is packed with silica or polymer beads with dimensions of ~10 micron; average pore sizes can range from 10 to 1000 nm.



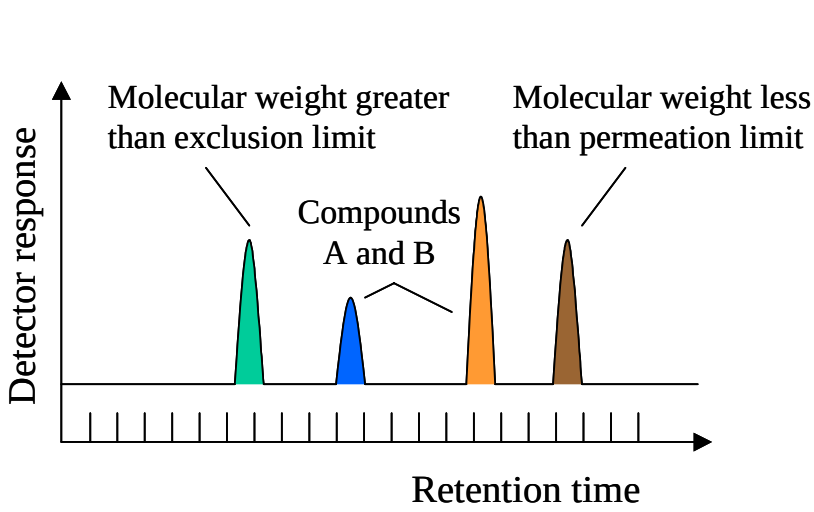
Path followed by molecule with dimensions greater than size exclusion limit (shortest transit time)

Path followed by partially permeating molecule (intermediate transit time)

Path followed by fully permeating small molecule with dimensions less than the permeation limit (longest transit time)

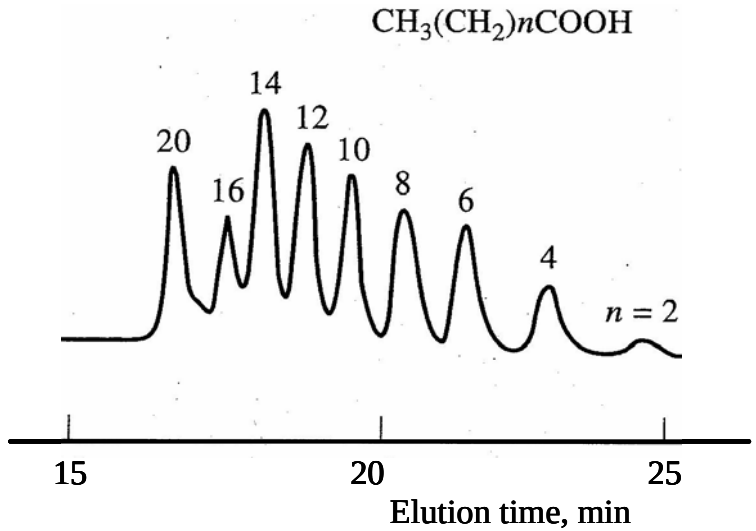
Detector Response

Output signal from an SEC column can be used to determine organic molecule concentration versus molecular weight for liquid samples



(a)

Typical detector response curve (adapted from Skoog, 1998, Principles of Experimental Analysis). For molecules with molecular weights intermediate between the size exclusion limit and the permeation limit, the detector response can be converted into a mass spectrum.



(b)

Size-exclusion chromatographic separation of fatty acids in a column packed with polystyrene beads (exclusion limit 1000 amu), obtained by DuPont Instrument Systems.

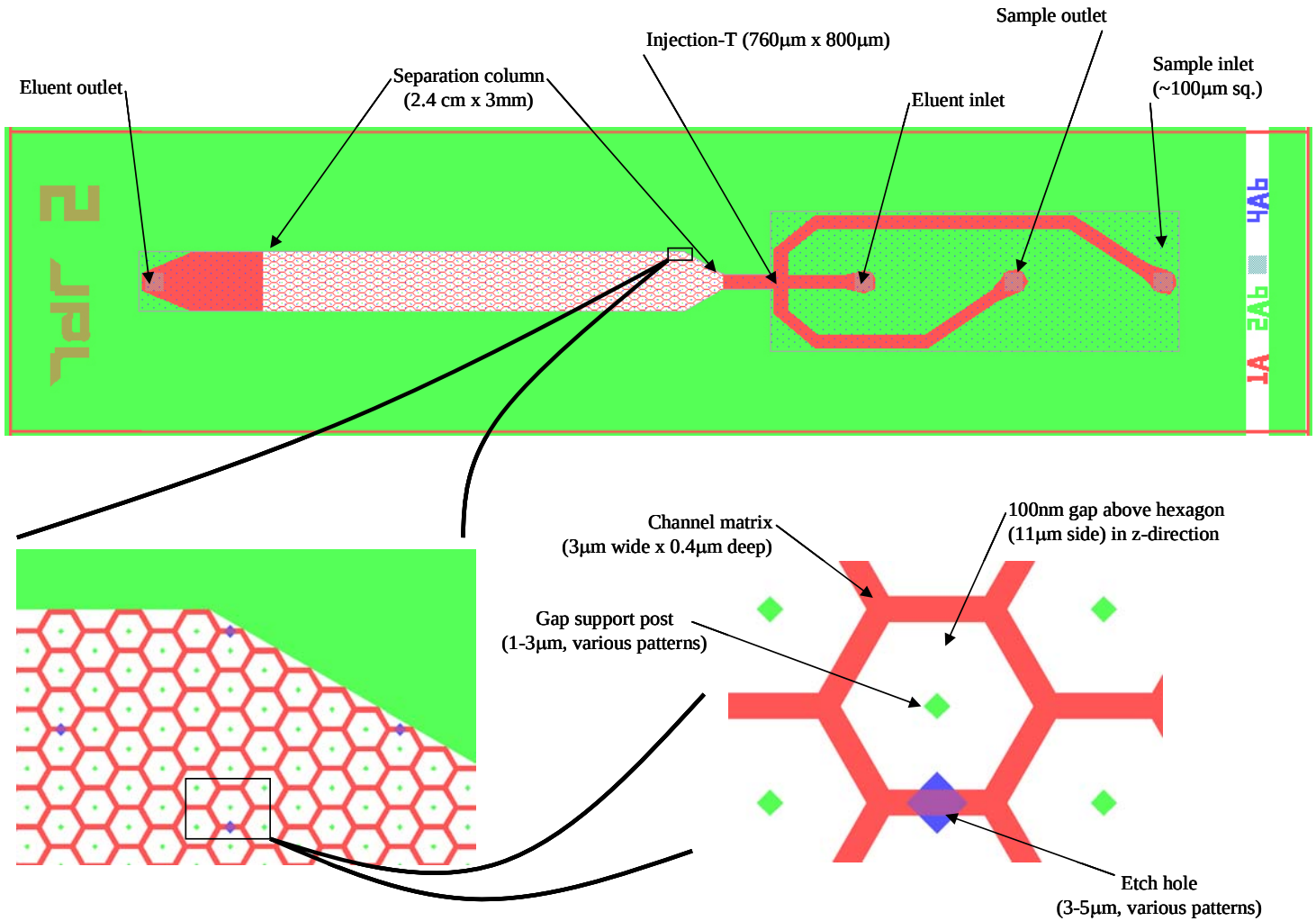
Advantages of n-SEC approach

Organic macromolecule separation using SEC requires only that molecules differ in diameter. Thus second-guessing with regard to missed classes of organic molecules is avoided. The nSEC offers significant advantages when compared with instruments designed to detect target classes of organic compounds because the nSEC can provide definite and interpretable information even if organic macromolecule development followed a different path on other planetary bodies.

Advantages of nano-SEC over conventional SEC are expected to include the following:

- ***Greater column reproducibility***
- ***Column packing (with associated voids) not required***
- ***Sub-nL injection volumes***
- ***Control over size exclusion separation parameters through manipulation of feature geometries in nanochannel network***
- ***Simplified injection using microfabricated high pressure electrokinetic pump***
- ***Separation column mass is reduced by orders of magnitude***
- ***Complete nSEC instrument is expected to be portable, robust, miniature (~2 kg), and operate on low voltages and low power (~ 5 W)***

NanoSEC Column Prototype



Fabrication sequence

A

(A) Growth of a 100 nm thick oxide layer on Si.

B

(B) Definition of 100nm tall post structures by CF4-RIE.

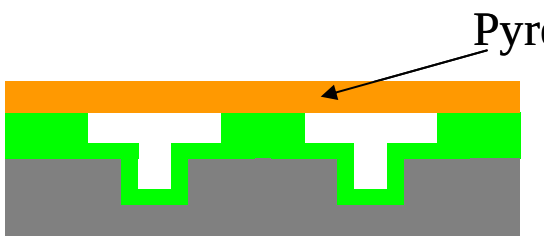
C

(C) Channel patterning by SF6-RIE.

D

(D) Growth of 10-20 nm thick thermal oxide layer.

E

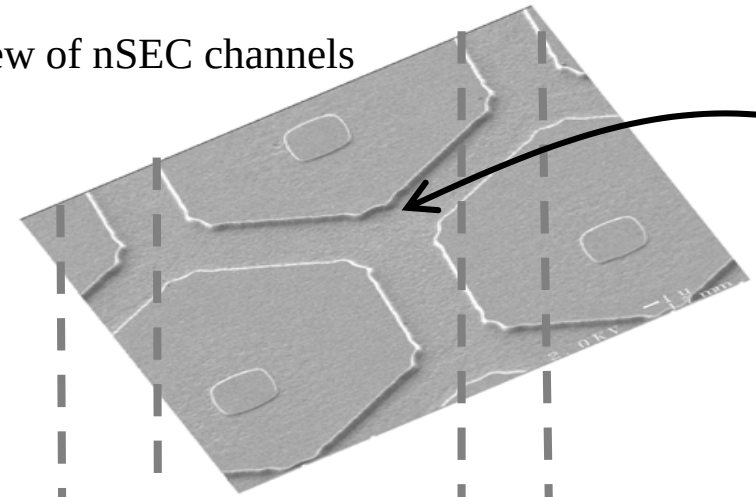


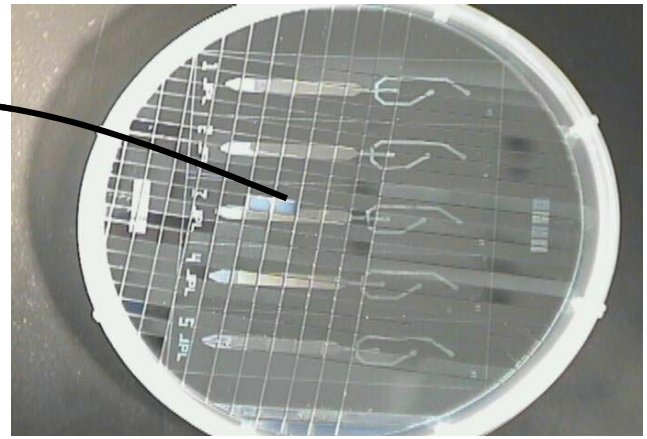
(E) Anodic bonding of a Pyrex cover wafer onto a channel wafer for sealing.

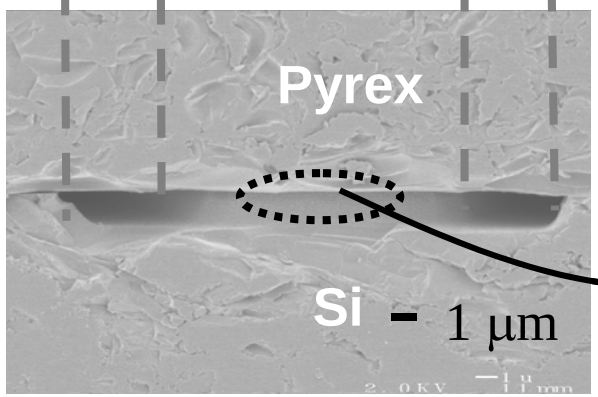
prototype device

Sealed Si wafer containing five nSEC columns

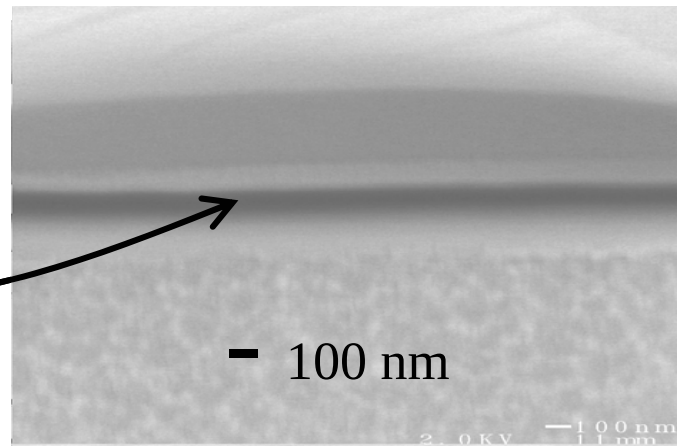
Top view of nSEC channels







Cross-section of bonded wafer showing 1 μm and 100 nm gaps

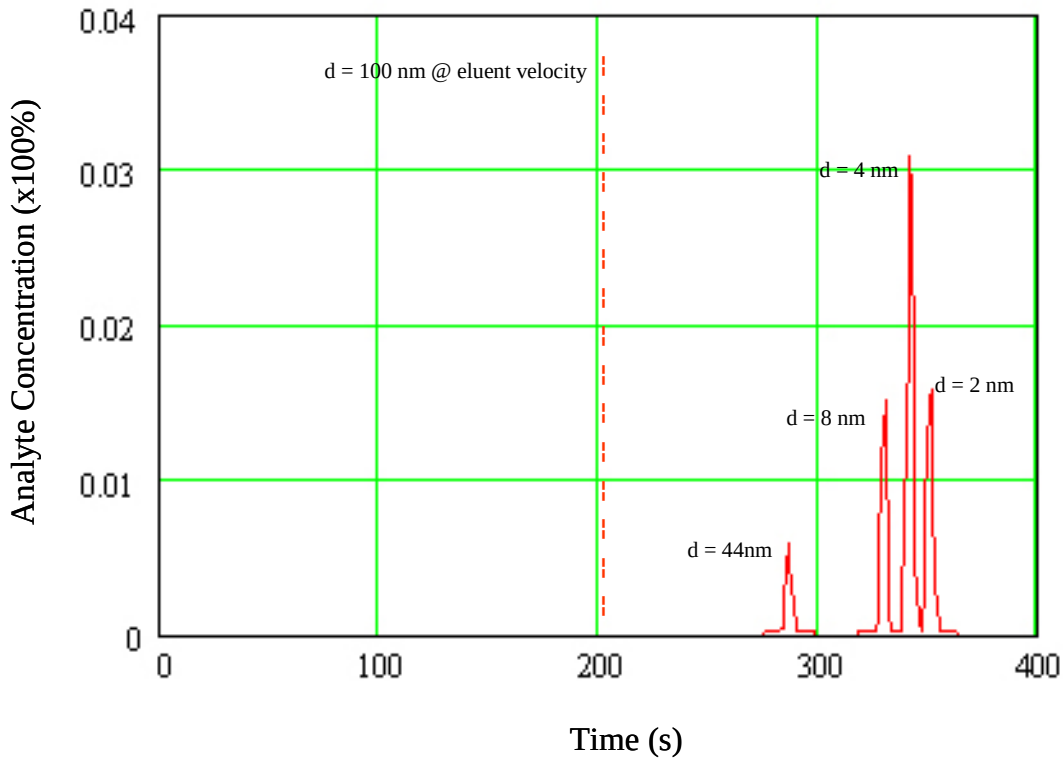


SEM image confirming presence of 100 nm gap in sealed nSEC device

Theoretical model

Assumptions:

- Hele-Shaw type fluid flow in parallel plates (constant pressure across separation channel)
- Flow rate varies as square of the gap height
- Analyte separation is achieved by diffusion of the analyte between a "mobile phase" (1 micron gap) and a "stationary phase" (100 nm gap)
- Negligible surface effects
- Flow rate of 0.0015 nL/min @ 55 psi



Chromatograph of Model Analyte

Theoretically predicted sample separation is highly dependent on molecular diameter, leading to efficient size exclusion separation.

Conclusions

- **Developed a prototype of a nano-fabricated size exclusion chromatograph system**
- **Developed a novel process of fabricating nanometer-size gap by microfabrication**
- **Nanofluidic experiments using fluorescent beads with a flow rate of 0.0015 nL/min in progress**

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