An Investigation into the "World-to-chip" Interface

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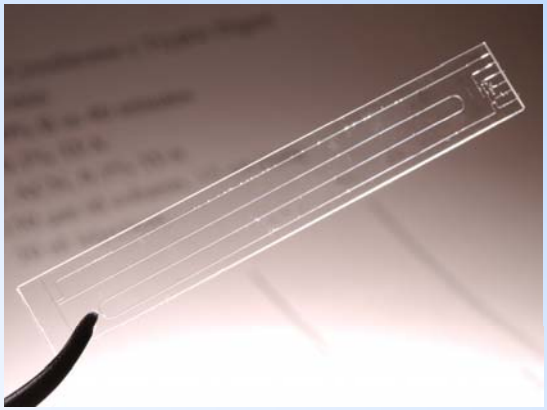
TFUG Meeting, San Jose, CA

HPLC on-a-chip

Overview

- Challenges of small scale chromatography
- Advantages of microfluidics
- Component development for LC on-chip
- Separations



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Shrinking an HPLC

Goal: Miniaturize HPLC and maintain performance → minimize system dispersion

$$\sigma^2 = \sigma_{col}^2 + \underbrace{\sigma_{inj}^2 + \sigma_{tube}^2 + \sigma_{fittings}^2 + \sigma_{det}^2}_{\sigma_{ext}^2}$$

Loss of separation efficiency from components

d = 1 mm	$\sigma_{ext}^2 = 45{,}300\ nL^2$
d = 0.3 mm	$\sigma_{ext}^2 = 370\ nL^2$
d = 0.1 mm	$\sigma_{ext}^2 = 14\ nL^2$

- Large injection volumes
- Connection tubing
- Fittings and connectors
- Detector cell

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Why Microfabricate?

Benefits of Microfabricated LC

- **Reduce number of system components**
- **Reduce human intervention**
- **Improve system-to-system reproducibility**
- **Increase performance**
- **Green operation**
- **Scalability of cost**
- **Expand capabilities of HPLC**
  - Sample handling and transfer
  - Portability
    - On-line reaction monitoring
    - Field-portable HPLC systems

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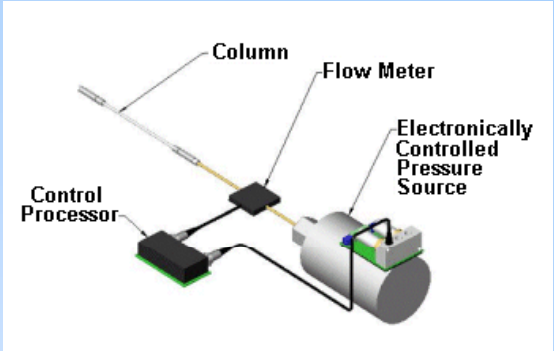
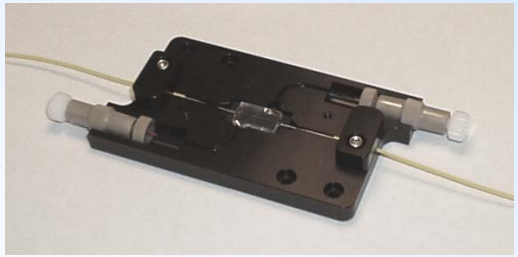
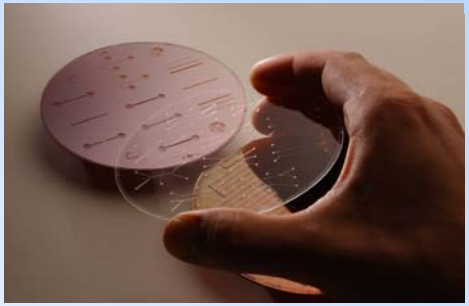
Required Technology

- Microfabrication
- Precision Fluid Delivery
- Detection
- Integration
- Chip-to-World Interface







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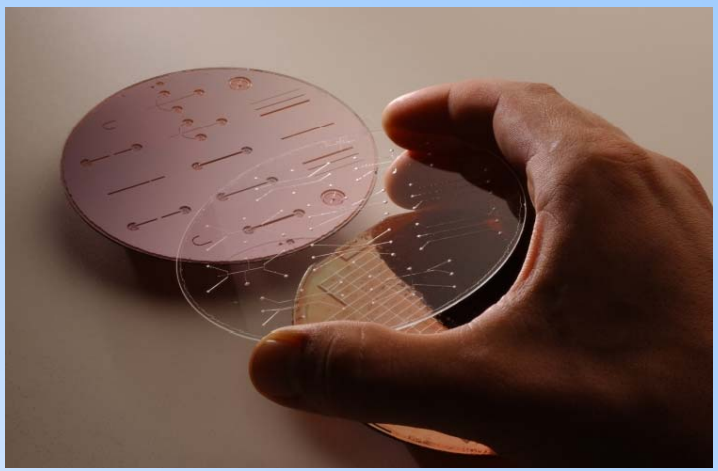
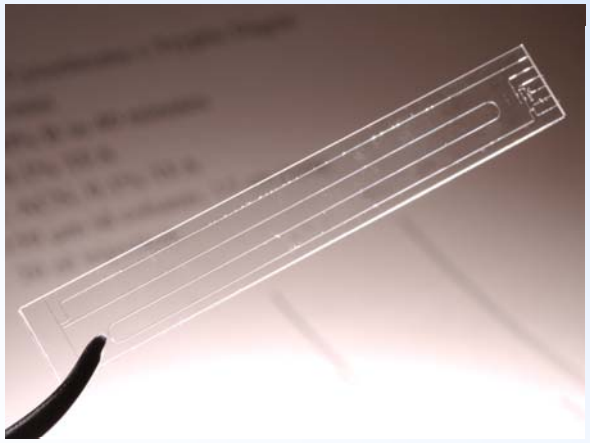
Microfabrication

- **High pressure compatible structures (>5,000 psi)**
- **Chemical compatibility**
- **High temperature operation**





- On-chip functionality:
  - –Valves (pL volumes)
  - –Injectors (precise 5 nL injections)
  - –Columns (variety of phases)
  - –Detectors (UV, Echem, MS, …)
  - –Connectors (high pressure, user-friendly)
  - –Sample Preparation (filters, traps, …)

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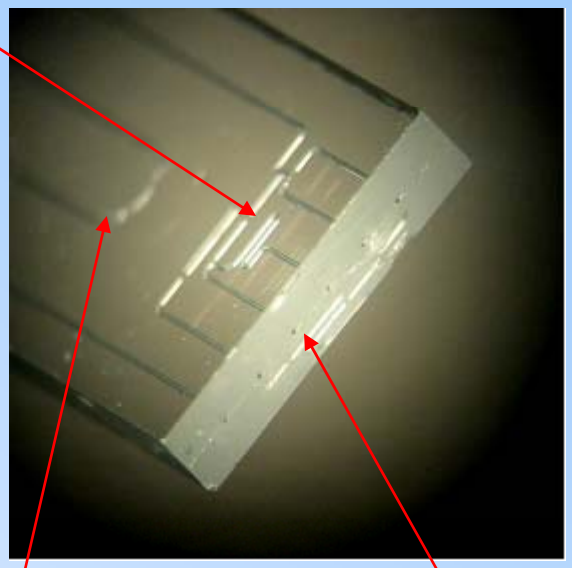
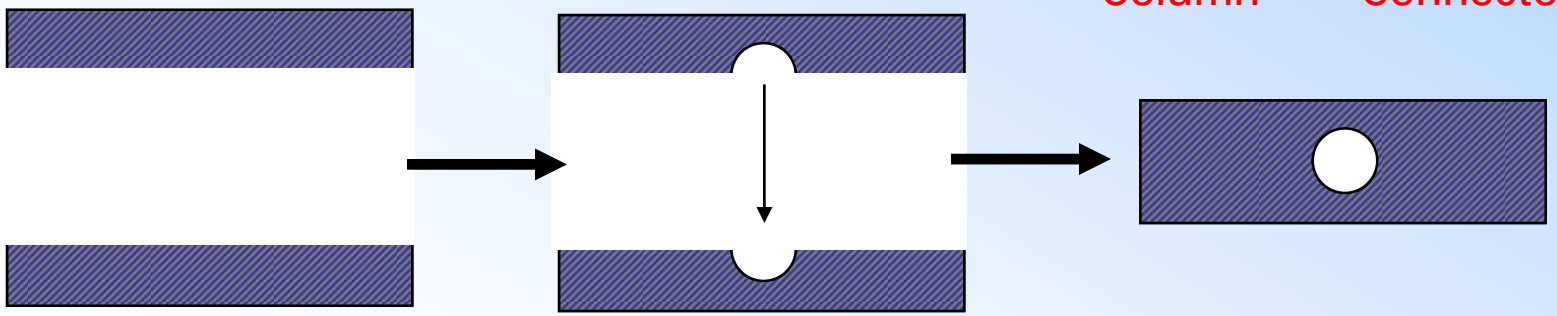
Chip to World Interface - Connectors

- Double-side etching in silica
  - **Cylindrical channels**
  - **Separation columns**
  - **Edge connectors**
  - **Injectors**
  - **Detection sensitivity**
  - **Overall system performance**
- Better pressure performance
  - **6000 psi routine**





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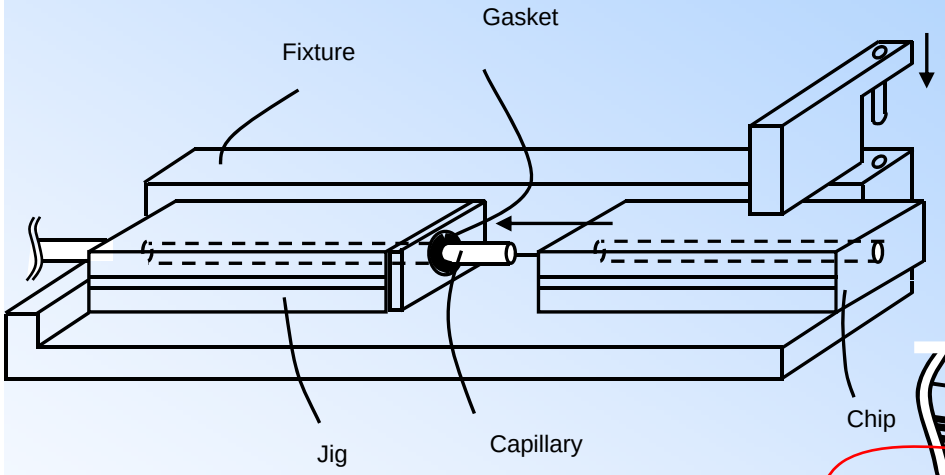
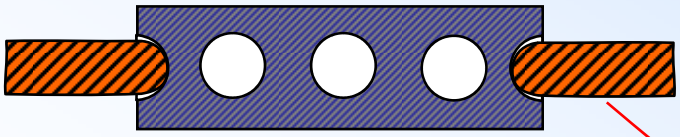
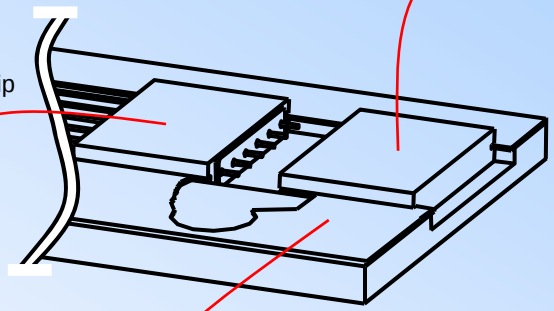
Connectors – Alignment

Lithography-controlled alignment of multiple simultaneous connections

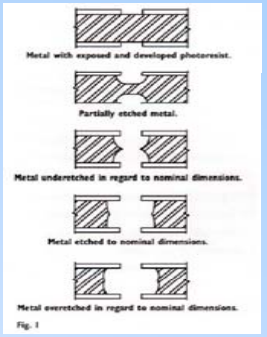






Chip

Jig



Chemically-Etched Shim Stock

D. W. Arnold, K. R. Hencken, P. P. Leung, S. S. Datwani, D. R. Cyr , J. E. Rehm.“Microfluidic Connections” US Application: # US2005056321A1; PCT Application: # WO2005096751A3; EP Application: # EP1740984A3; CA Application: # 2561508A1.

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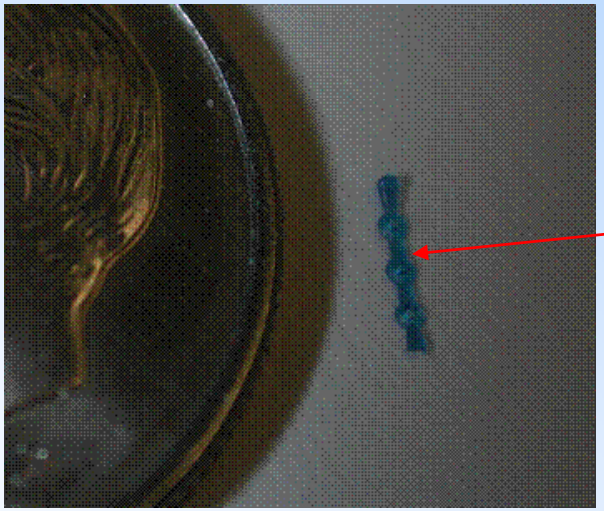
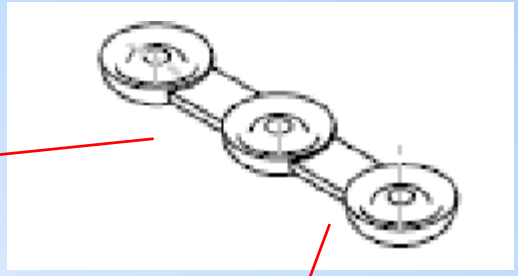
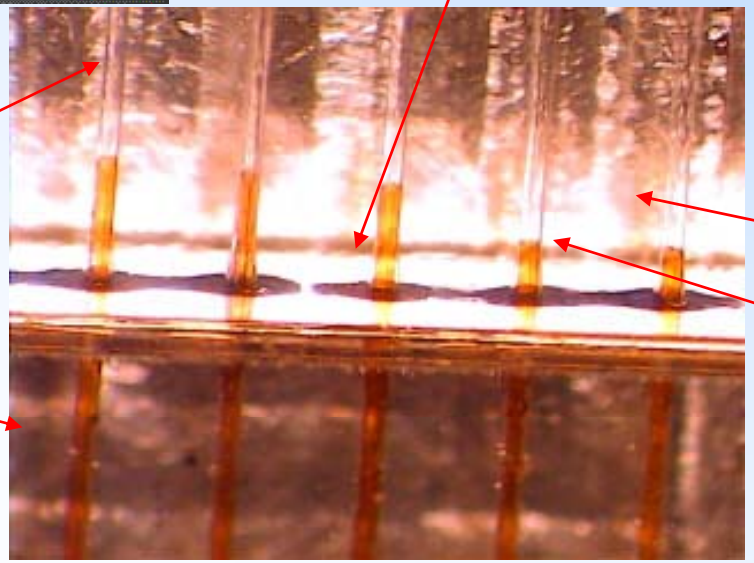
Connectors – Micro injection molded gasket







Material: Polypropylene for mechanical and chemical properties

Critical Dimensions: 150 um through vias on 1 mm pitch

Two per 6-port connection

Brass shim stock for retainer

Excellent performance

Fluidic channel

Working fluidic chip

Jig chip

capillary

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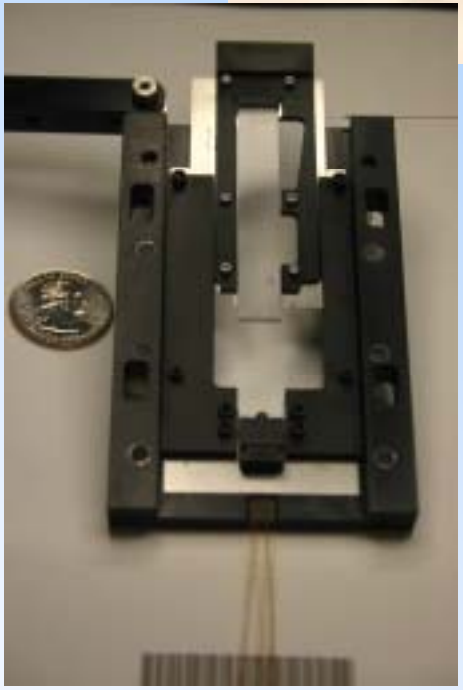
Connectors – Assembly performance

Robust connection for parallel channels to outside world

Excellent performance
- > 7200 psi demonstrated
- 50+ make/breaks for 6 connections to 4500 psi
- Microcantilever springs for added alignment precision - excellent
- Chip caddy
- Micro-injection molded gaskets and lithographically defined retainers

Minor issues with chipping of parts being addressed.



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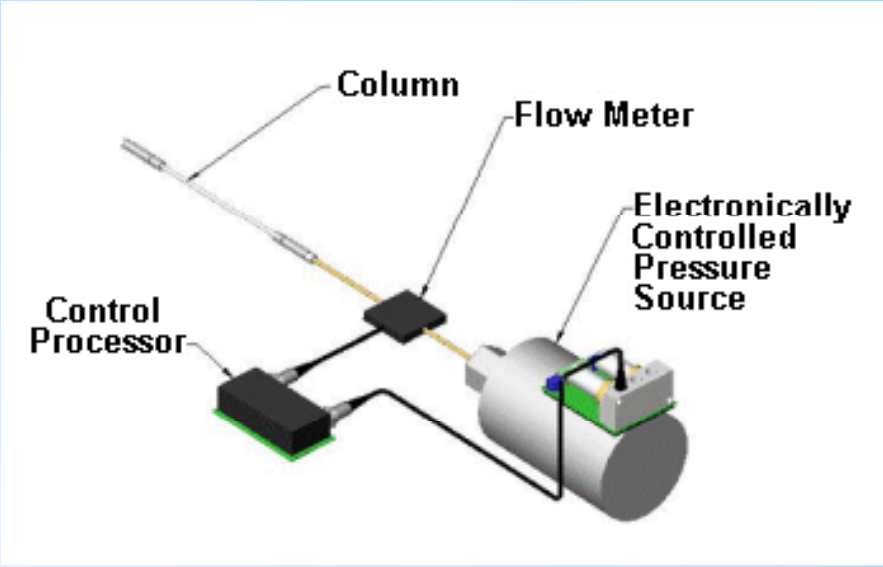
Precision Fluid Delivery

Microfluidic Flow Controller (MFC)

- Active Flow Control - feedback control on rapid, electronically adjusted variable pressure source
- Flow meter measures the flow rate (nL/min to 10s of µLs/min)
- Flow rate is maintained regardless of system back pressure or viscosity
- High performance, reliable microscale flow source
- Fast, reproducible gradients



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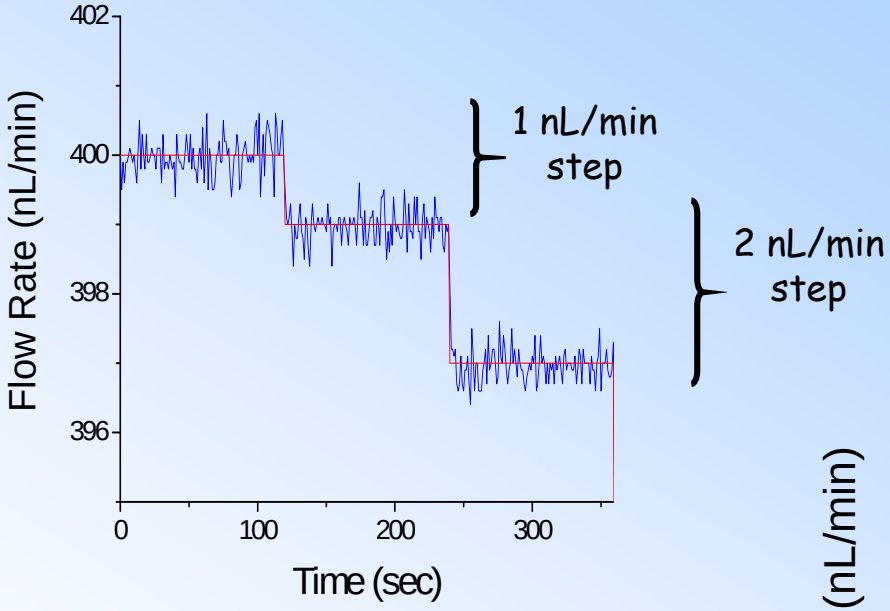
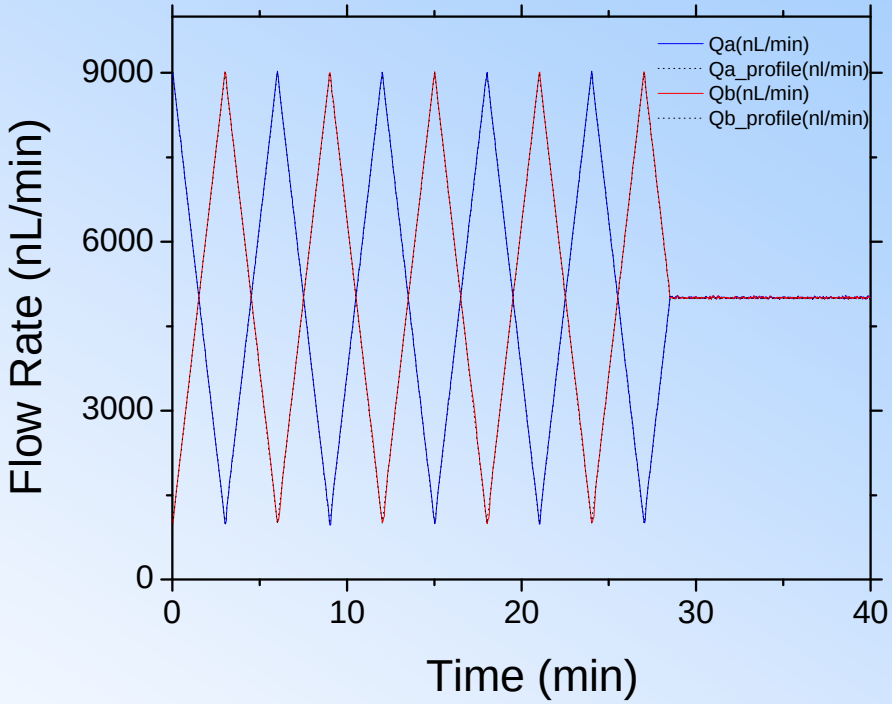
Microfluidic Flow Controller





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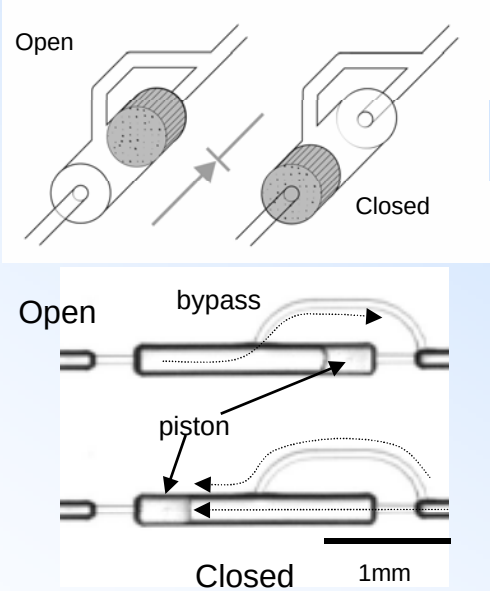
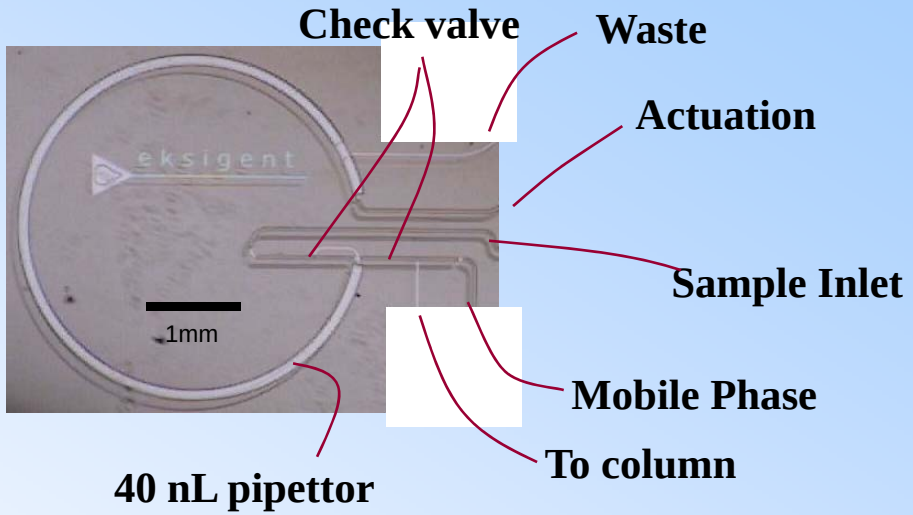
Microfabricated Sample Injector

Concept Example - Check Valve



High Pressure injector –

Two check valves and a pipettor



J. E. Rehm, T. J. Shepodd and E. F. Hasselbrink, MicroTAS 2001 Proceedings, pp. 227-229

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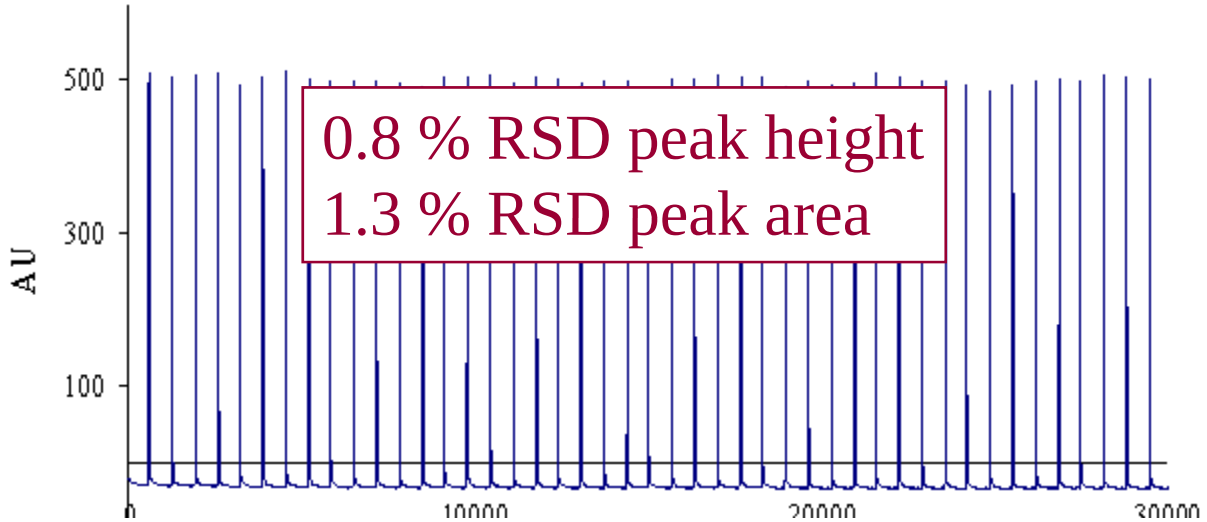
Injector performance

High-pressure operation (6000 psi)
Excellent chemical compatibility
Excellent reproducibility (manufacture and operation)



40 nL injections; 10 mM Thiourea (in MeOH)
200,000 injections; on shelf for 6 months; **200,000** injections
Detection: 239 nm

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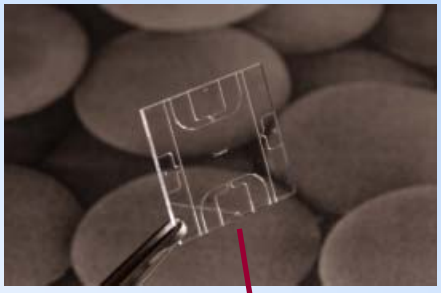
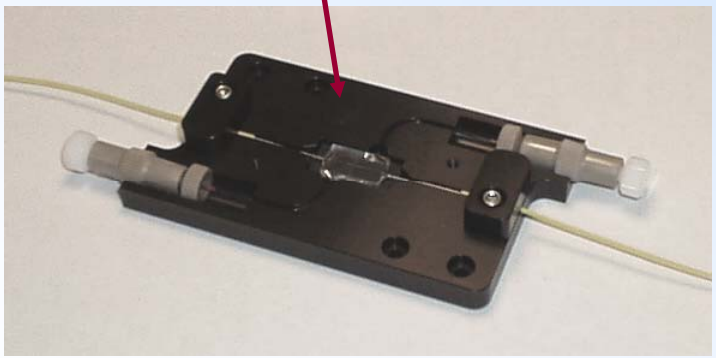
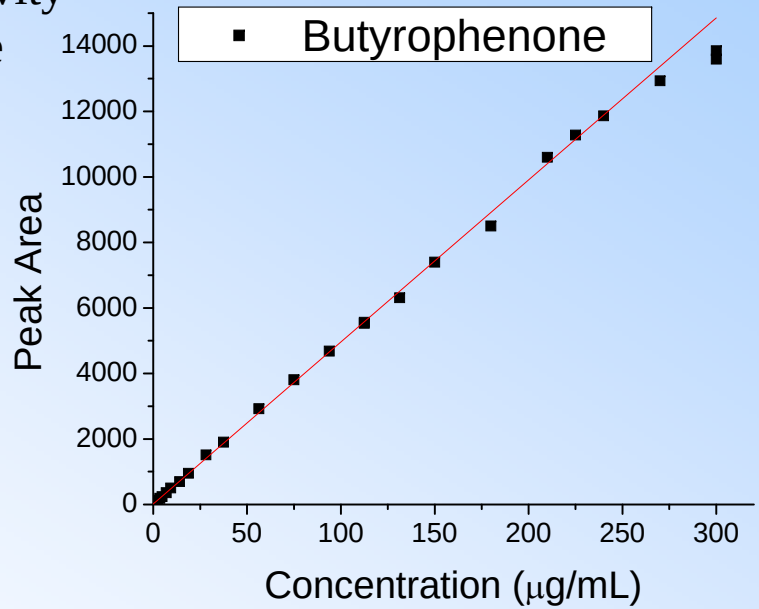
Detection – UV Absorbance



Microfabricated UV Flow Cell (silica)

- Fiber optic illumination/collection
- 45 nL cell volume
- 4 mm path length
- Standard cell for ExpressLC® systems
- Improved versions under development
  - Higher sensitivity
  - Lower volume





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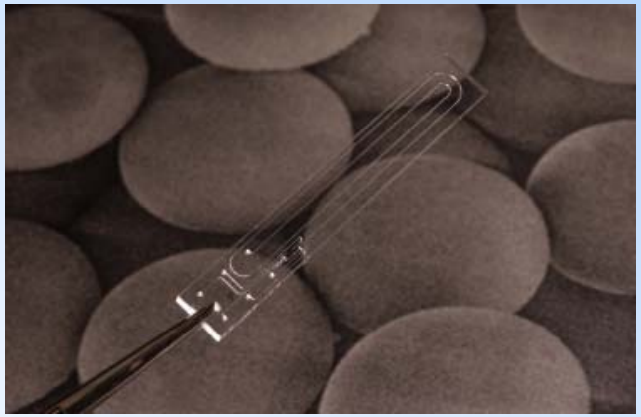
Columns

- Columns microfabricated with 125, 150, 200 and 300 µm diameters
- Particles retained by microfabricated and/or synthesized frits
- Conventional separation particles
    - Various particle manufacturers, phases, sizes
    - Method flexibility
    - Facilitate method transfer to microfabricated system
- Easy cHiPLC™-to-conventional LC comparison
- Temperature control facilitated by low thermal mass and device geometry
- Isocratic and gradient methods



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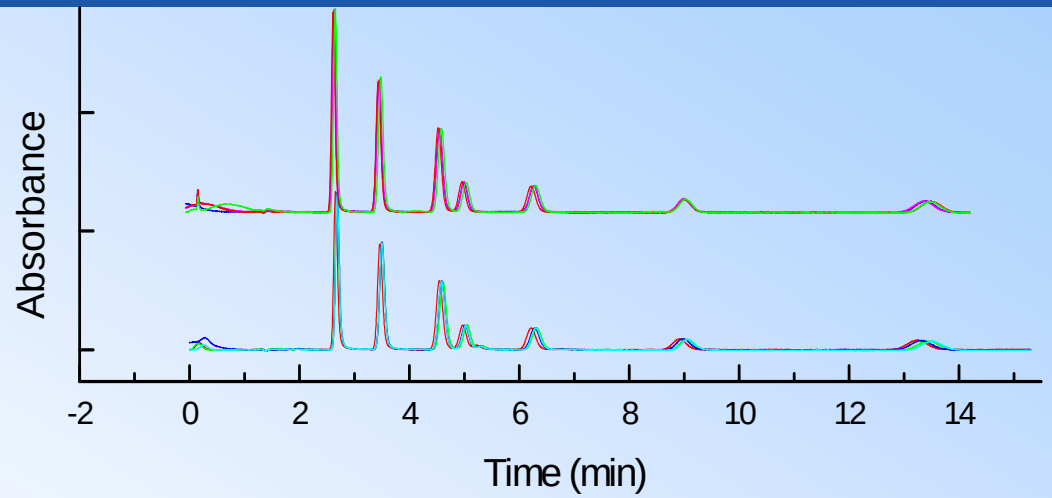
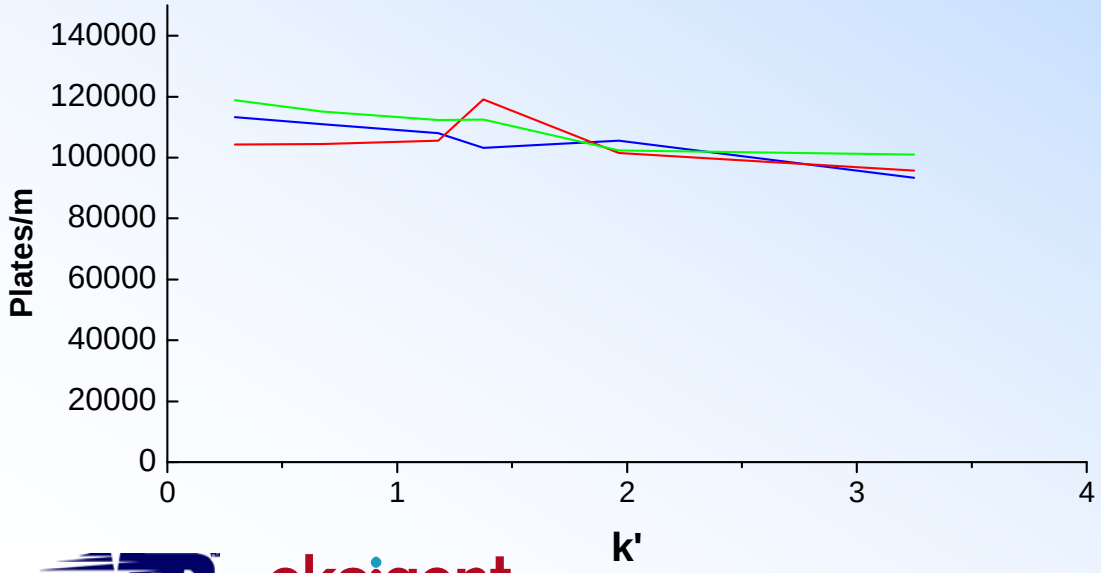
Column-to-column performance





3 columns
4-5 replicate runs each
25 nL injections
(cHiPLC™ injector)
Phenone mix

Separation conditions:
200 µm x 6.2 cm column
3 µm Luna C18
1 µL/min
Isocratic 40 % H_2O : 60 % ACN
Detection: 240 nm

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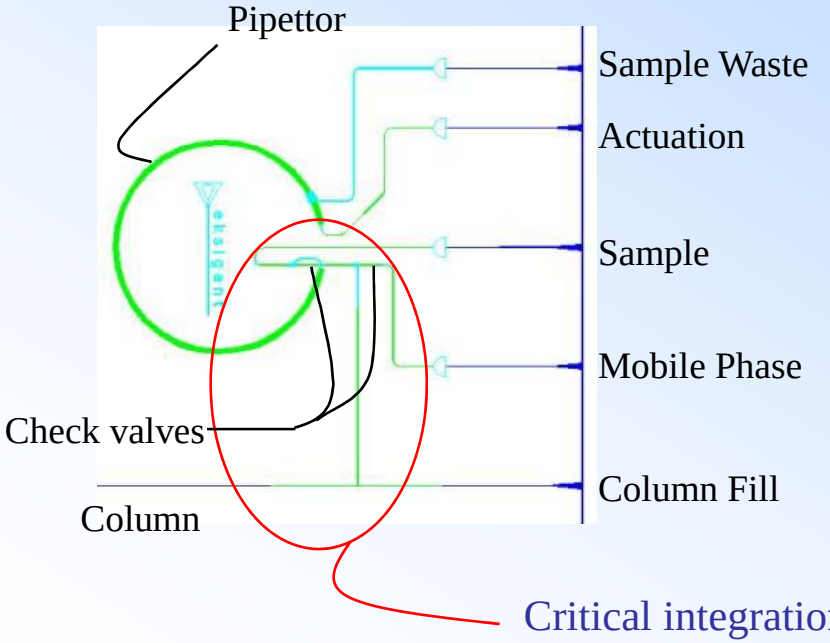
Integrated Inj-Col-Det

Column Diameter	Flow Rate (µL/min)	Injection Volume (nL)	Detection Volume (nL)
150 µm	1.0+	10	10
200 µm	1.8+	20	20
300 µm	3.5+	40	40

•150 mm – Detection volume too small
•300 mm – Serpentined columns have too much dispersion

•200 mm – Preferred balance of integrated system characteristics



- No connections in critical analysis region
- Low volume / small dimensions around turn
- Gentle expansion into larger diameter region of column
- Balance dispersion and pressure drop

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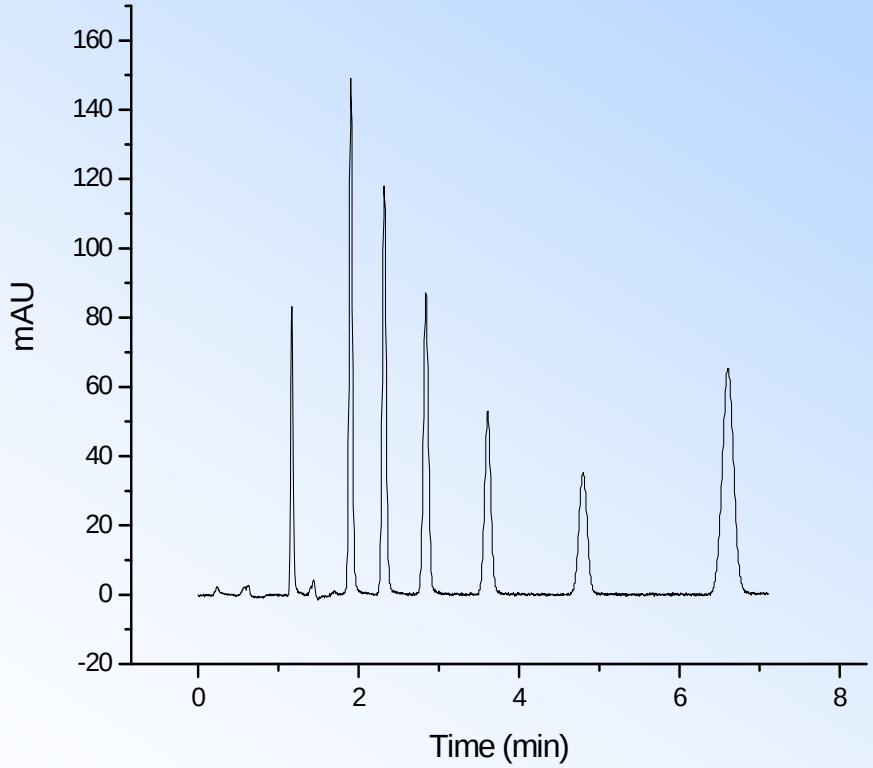
Integrated Microfabricated System



200 µm x 10 cm column
3 µm Luna C18
1.2 µL/min
Isocratic - 30 % H_2O : 70 % ACN
20 nL injection
Detection: 245 nm

Compound	Theoretical Plates/m	Symmetry Factor
Uracil	**75030**	**0.84**
Acetophenone	**99660**	**0.94**
Propiophenone	**103580**	**0.94**
Butyrophenone	**101050**	**0.97**
Valerophenone	**98260**	**0.99**
Hexanophenone	**97030**	**1.00**
Heptanophenone	**96420**	**1.00**

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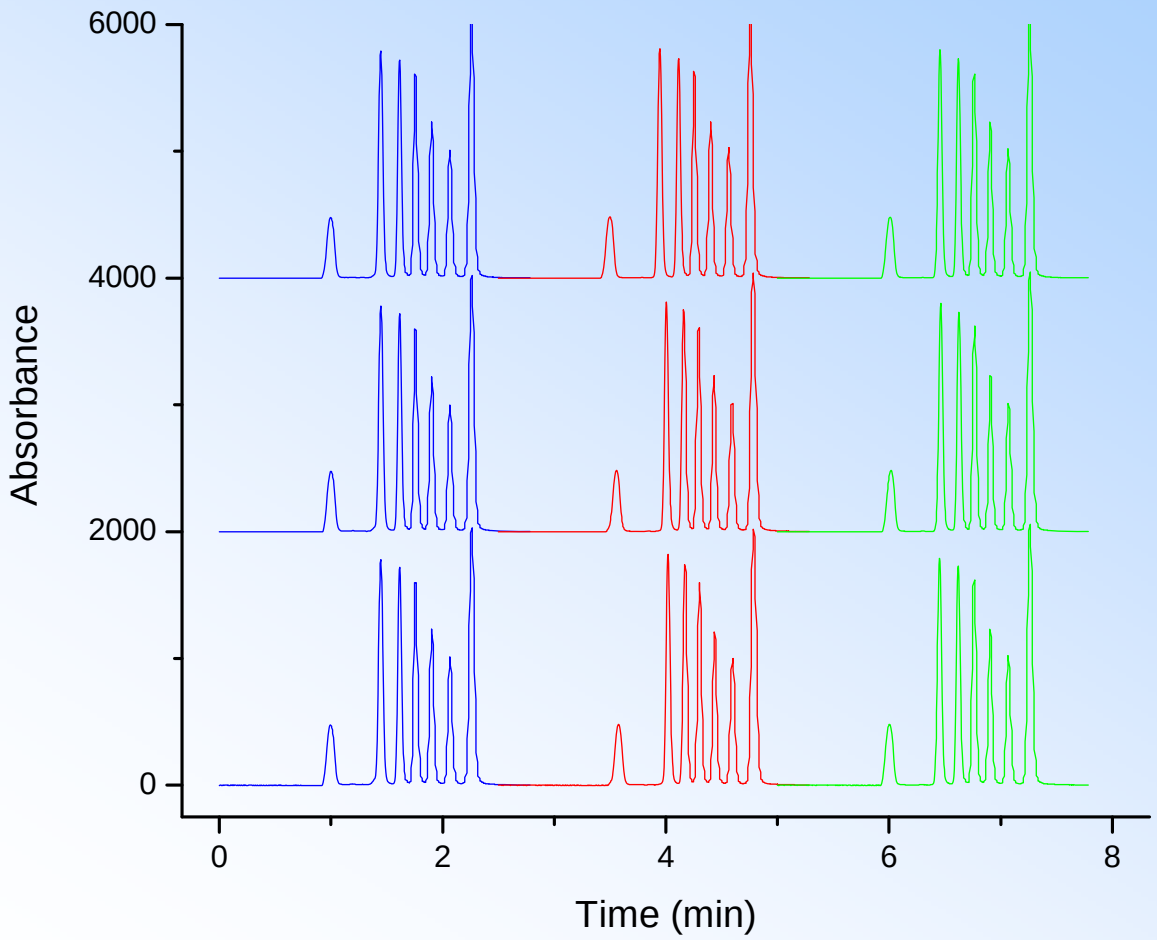
Reproducibility of Gradient Separations



Peak 3
0.7 % RSD peak height
0.8 % RSD peak area
< 0.1 % RSD retention time

Peak 7
0.7 % RSD peak height
0.9 % RSD peak area
< 0.1 % RSD retention time

A: H2O ; B: ACN
%B – 90%-100%; 6 sec
200 µm x 10 cm column
3 µm Luna C18
1.2 µL/min
20 nL injection
Detection: 245 nm

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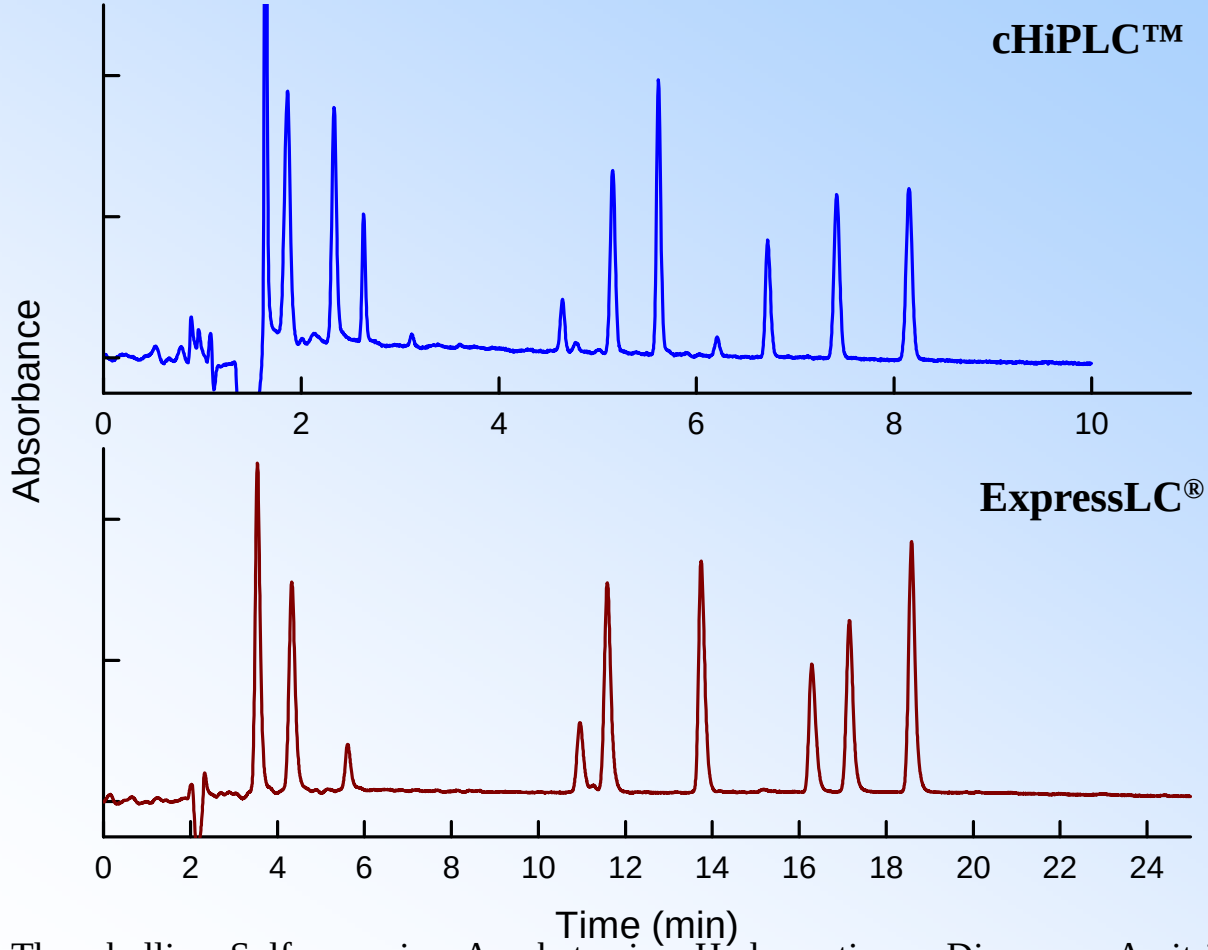
Test Sample Mix



A: 0.1%TFA/H_2O
B: 0.085% TFA/ACN

Gradient: 0-30 min
%B – 10%-90%

200 µm x 6.2 cm cHiPLC™ column
3 µm Luna C18
25 nL injection
2 µL/min
Detection: 214 nm

A: 0.1%TFA/H_2O
B: 0.08% TFA/ACN

Gradient: 0-30 min
%B – 10%-90%

300 µm x 15 cm Eksigent® column
3 µm Luna C18
40 nL injection
4 µL/min
Detection: 210 nm

Theophylline; Sulfamerazine; Amphetamine; Hydrocortisone; Diazepam; Amitriptyline; Bumetanide; Thioridazine; Fenoprofen

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Acoustic Droplet Ejection

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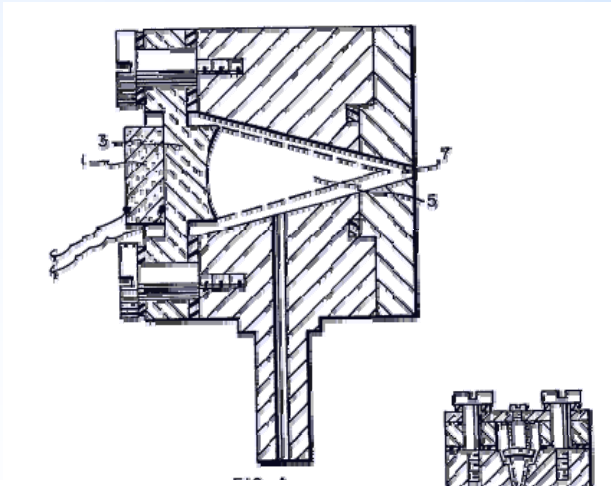
History of Acoustic Dispensing

- 1920's: No focus at surface
  - Uncontrolled drop size and trajectory
- 1973: Focus at surface – IBM
  - Controlled drop size and trajectory



IBM TECHNICAL DISCLOSURE BULLETIN, Sept. 1973, p. 1168

- **1979: Recognition Equipment Inc.**
  - Document printing applications
- **1985: Xerox Corp.**
  - Document printing applications
- **1993: NASA**
  - Part manufacturing
- **2000: Picoliter Inc.**
  - Life science applications
    - Acoustic dispensing
- **2005: Labcyte LLC/Picoliter Inc. merger to form Labcyte Inc.**
  - Instruments offered as well as acoustically qualified consumables

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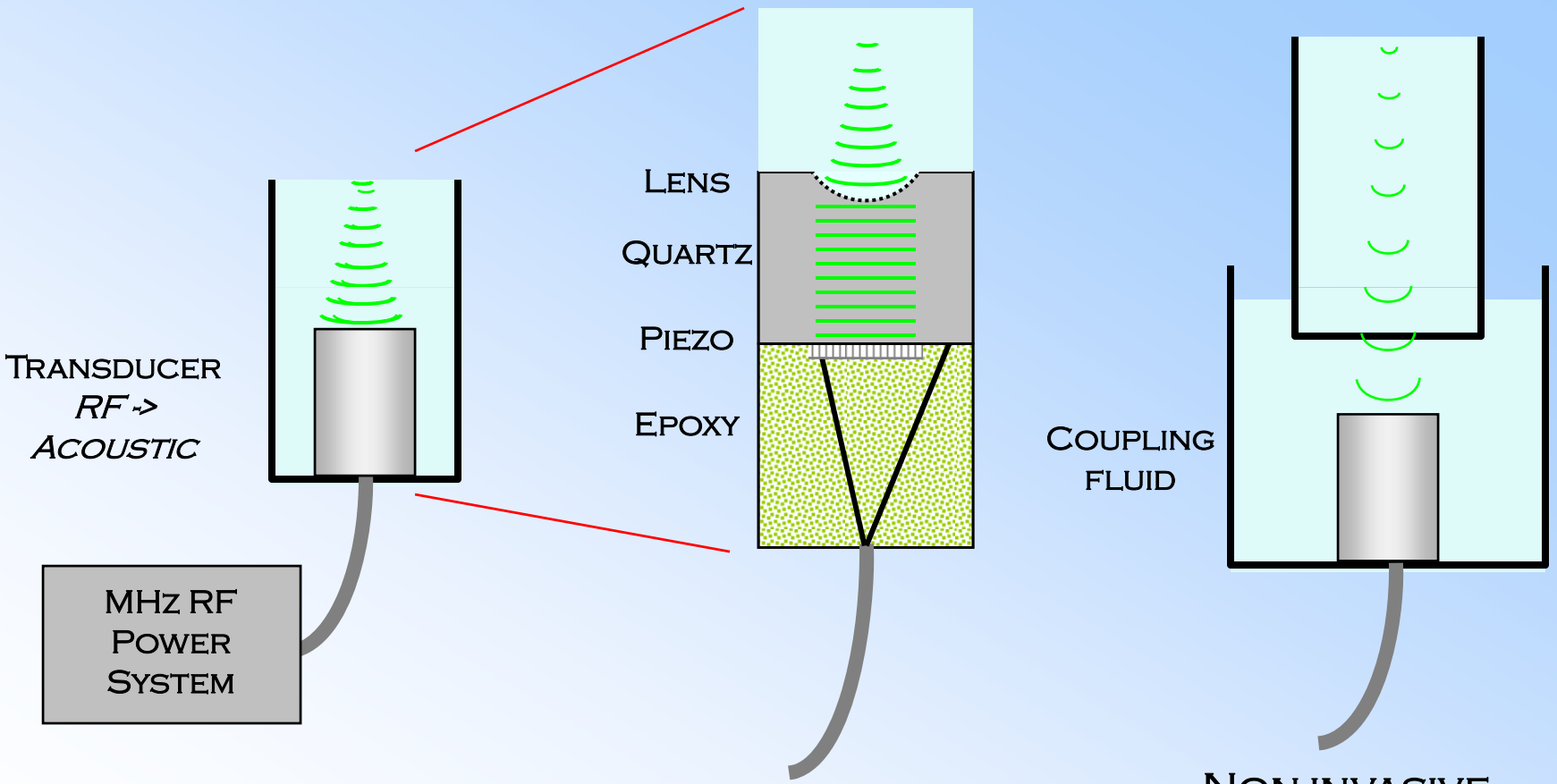
Non-invasive Acoustic Ejection



Immersion

Non-invasive (via "coupling fluid")

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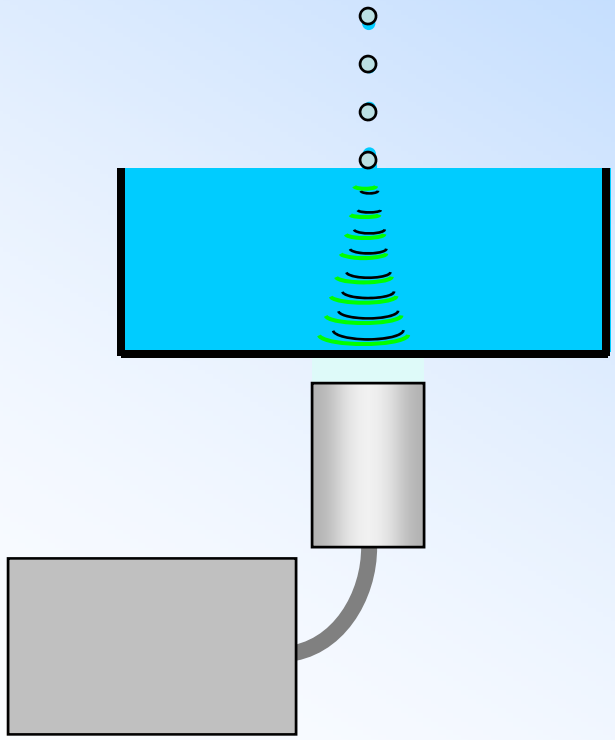
Drops



- **Drop volume is tunable**
  - inverse of RF frequency

    1 MHz ⇒ 200 nL

    100 MHz ⇒ 10 pL
- **Multiple drops for larger volumes**
- **Fluid properties have secondary influence over drop volume**

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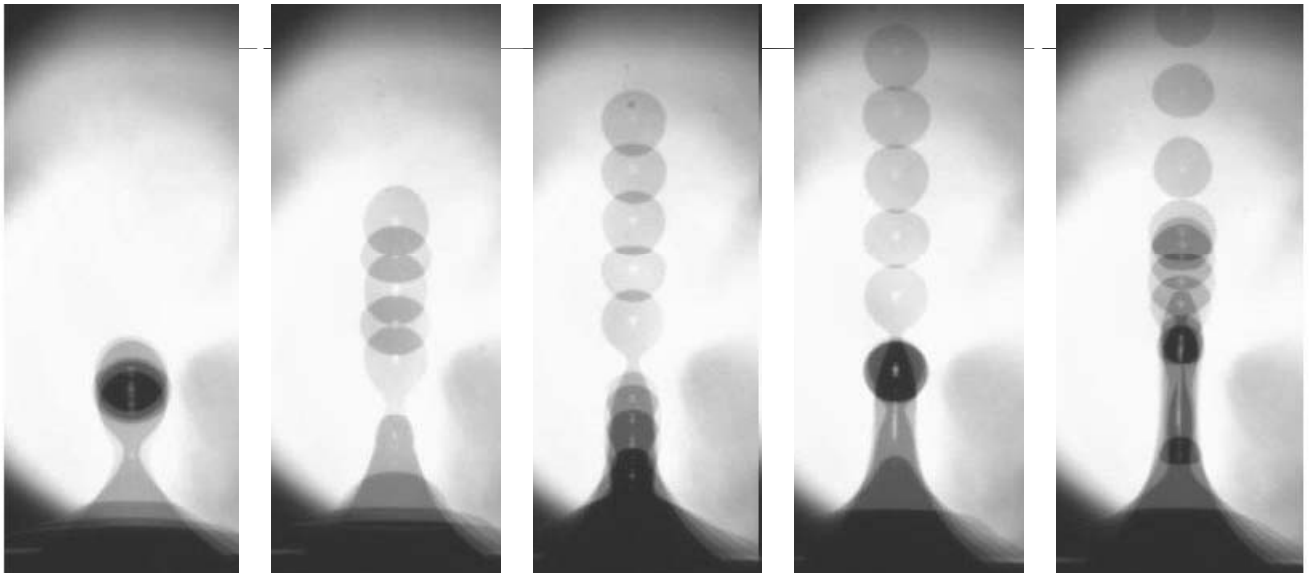
Single Ejection Event, Multi-Strobe Capture

- **Water Ejection**
  - 5 MHz



Low Power → High Power

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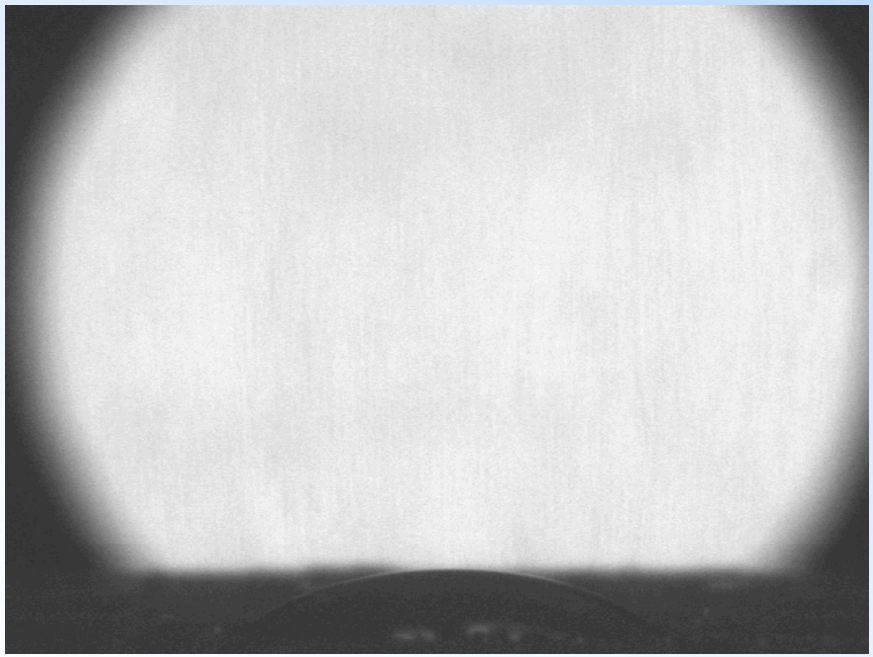
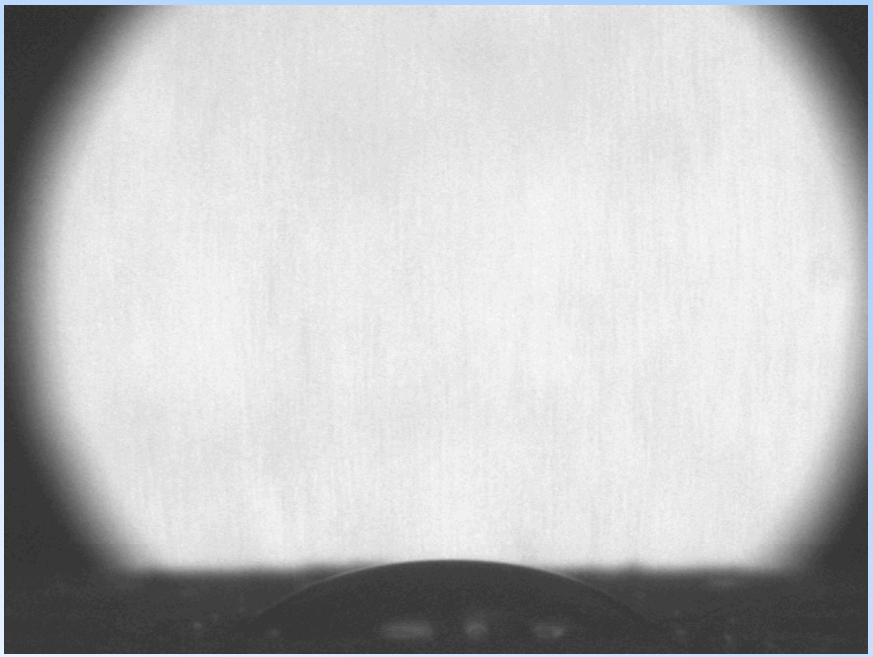
Changing Rep. Rate



750 Hz



1180 Hz

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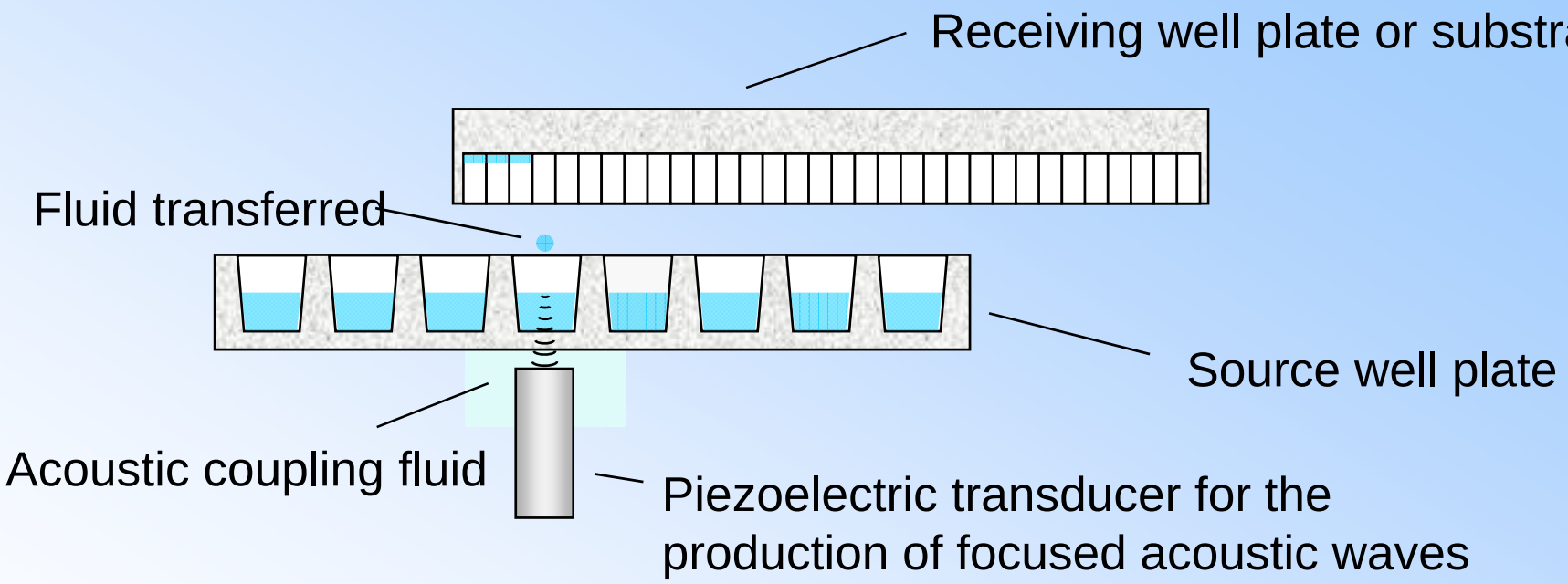
Well Plate to Well Plate Transfer



- **Well plates used: Greiner, Nunc, Whatman, and Corning Costar**

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Sample Transfer Results

			Well Volume						
Plate (hyperlinked to graphs)	Ramprint	Comments	Avg(nL)	sdP(nL)	CV	Min.(nL)	Max.(nL)	N	Total Wells
P070_5_787_001			5.04	0.13	2.64%	4.75	5.60	383	384
P070_50_787_002			50.70	1.42	2.80%	48.04	55.49	383	384
P075_5_787_003			5.04	0.17	3.31%	4.72	7.13	384	384
P075_50_787_004			50.53	1.40	2.77%	48.02	59.97	384	384
P080_5_788_001			4.99	0.14	2.79%	4.68	5.84	384	384
P080_50_788_002			49.94	1.20	2.40%	47.38	55.62	384	384
P085_5_788_003			4.97	0.15	2.98%	4.65	5.48	384	384
P085_50_788_004			49.63	1.30	2.61%	45.87	53.91	384	384
P090_5_788_005			5.07	0.16	3.19%	4.46	5.53	384	384
P090_50_788_006			50.78	1.47	2.89%	46.64	54.99	384	384
P095_5_788_007			5.12	0.19	3.63%	4.64	5.64	383	384
P095_50_788_008			51.47	1.63	3.17%	46.45	55.86	383	384
P100_5_788_009			5.09	0.15	2.92%	4.44	5.45	382	384
P100_50_788_010			50.94	1.35	2.65%	46.21	54.06	382	384
all plates			27.81	22.79	81.95%			5368	5376

- DMSO 100%, 95%, 90%, 85%, 80%, 75% and 70%
- Various fill heights
- 5 nL and 50 nL transferred with fluorescein
- PE EnVision fluoresence reader (Excellent Linearity)

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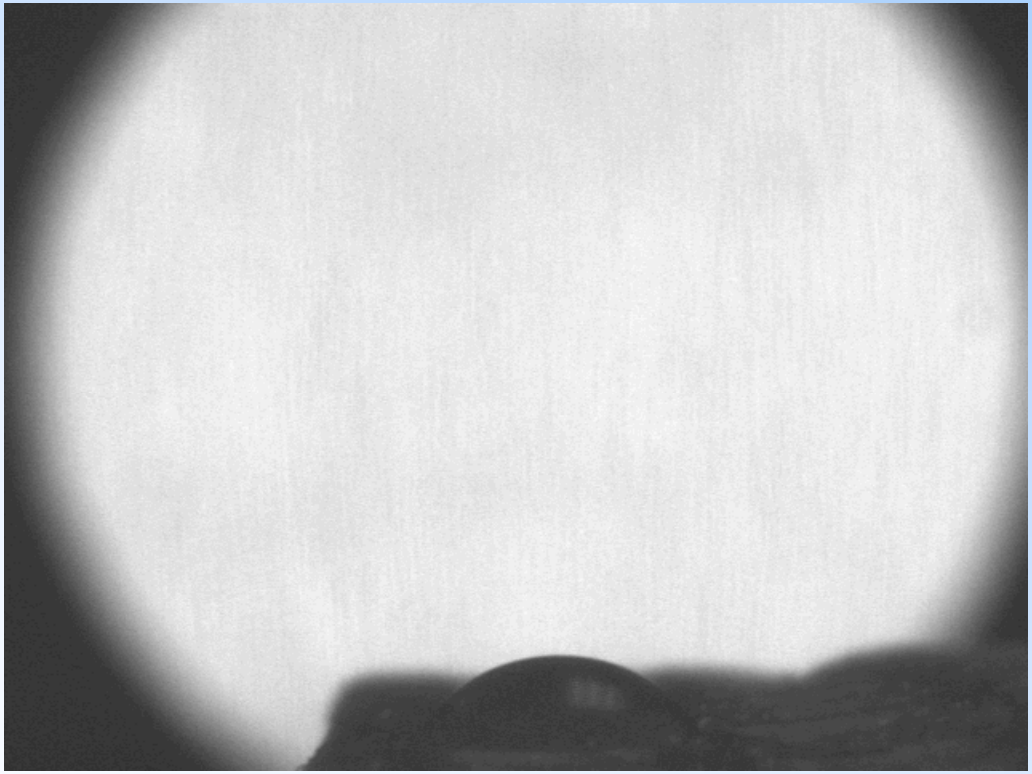
Drop Ejection from a 1536 Well Plate



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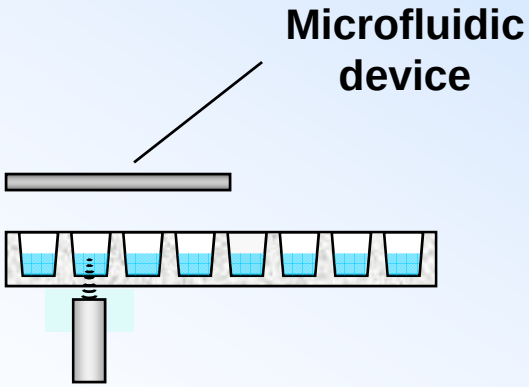
Microfluidic Device Fluid Transfer

Loading diversity:

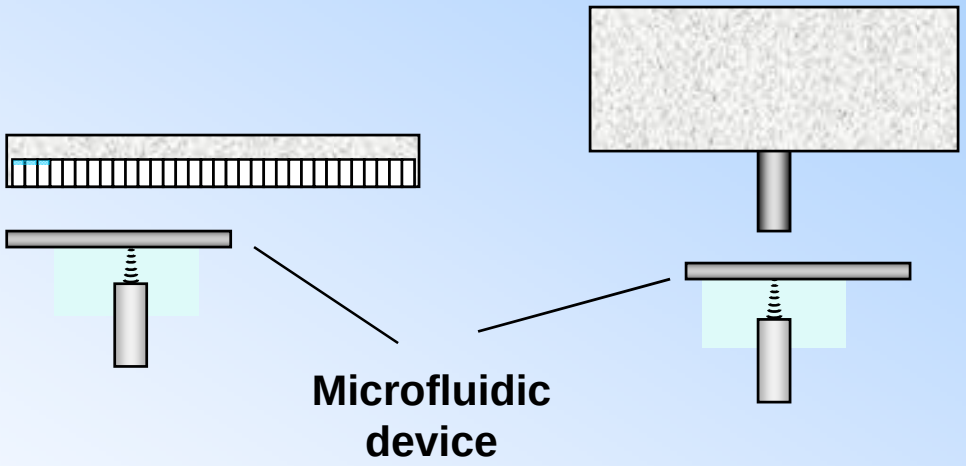
Well plate to microfluidic device



Unloading processed materials:

Microfluidic device to well plate

Microfluidic device to analytical instrument



- **What do microfluidic device “inlets” and “outlets” look like?**

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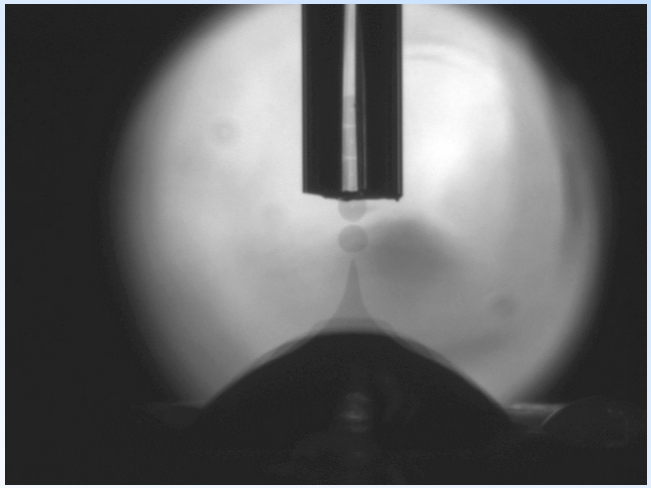
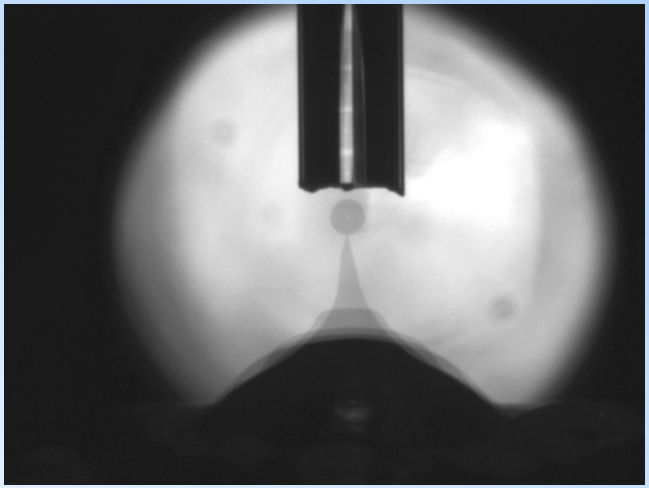
Drop Ejection into a Pipette

Water ejection into 1550 μm hole of Pasteur pipette

- Outer diameter 1840 μm



9.2 nL
7 MHz



19.2 nL
5 MHz

Images captured with multiple strobe flashes of a single droplet event.

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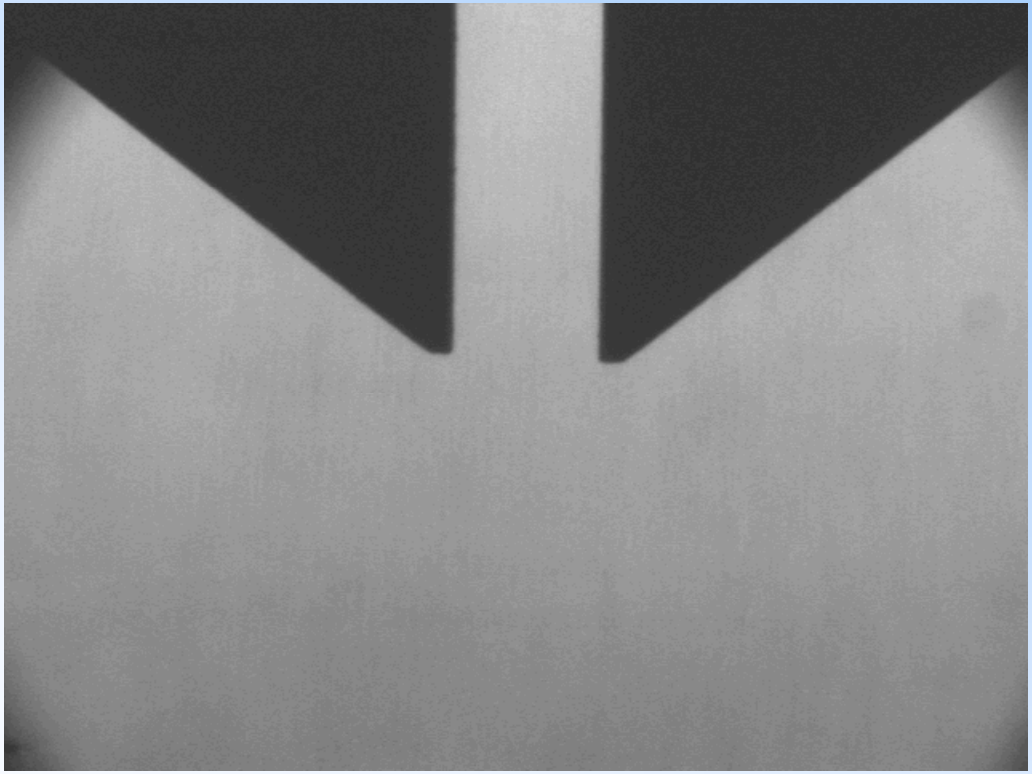
Drop Ejection into a Caliper



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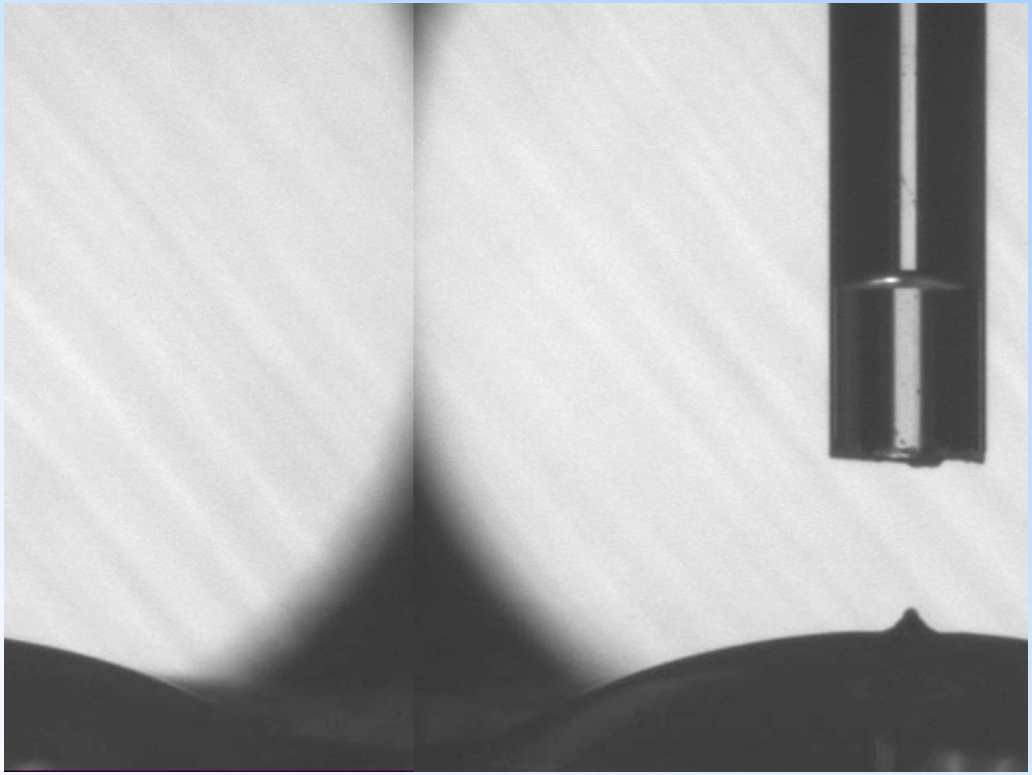
Drop Ejection into a Capillary



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Materials we can transfer

- **What can we transfer?**
  - DMSO (100% - 0%)
  - Aqueous solutions
    - Water
    - Salt solutions, buffers
    - Surfactant solutions (Triton X-100, SDS)
    - DNA, Primers, Probes
    - Proteins (BSA, Lysozyme), Fetal Calf Serum
    - Glycerol
    - Alcohol
  - Polymeric solutions
    - Acrylamide
    - Others
  - Ionic Liquids

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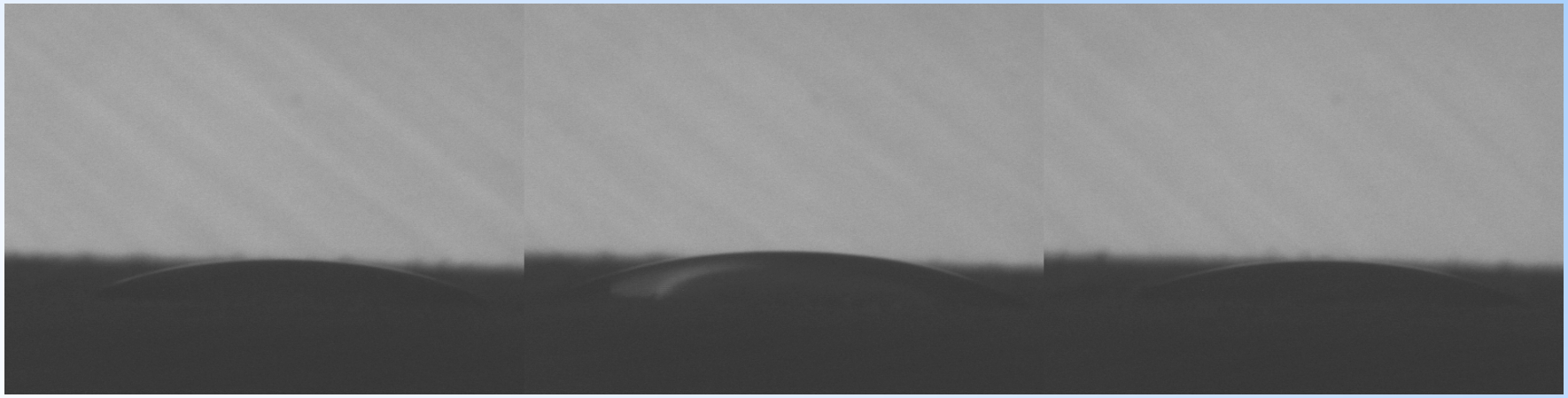
Low Surface Tension Fluids



Ethanol	0.005% Triton X-100	Water
γ = 25 mN/m	γ = 45 mN/m	γ = 73 mN/m

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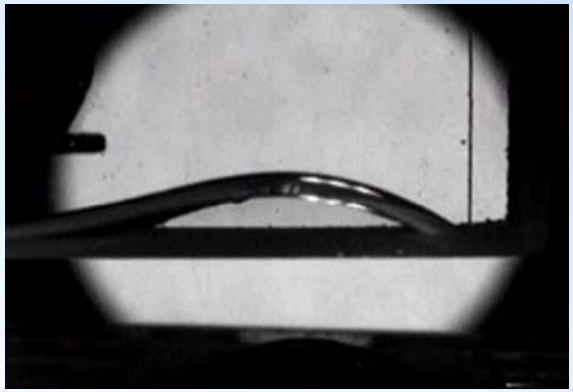
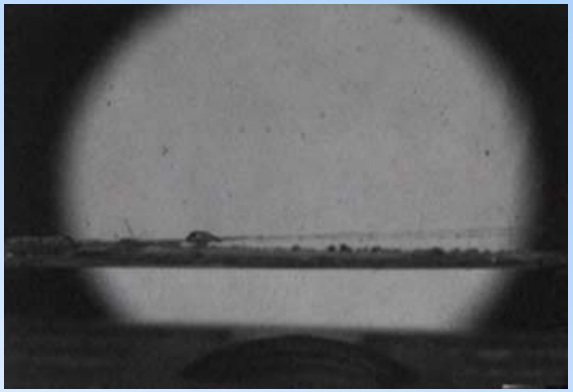
Acrylamide/ Mixed Fluid Systems



Less Dense



More Dense

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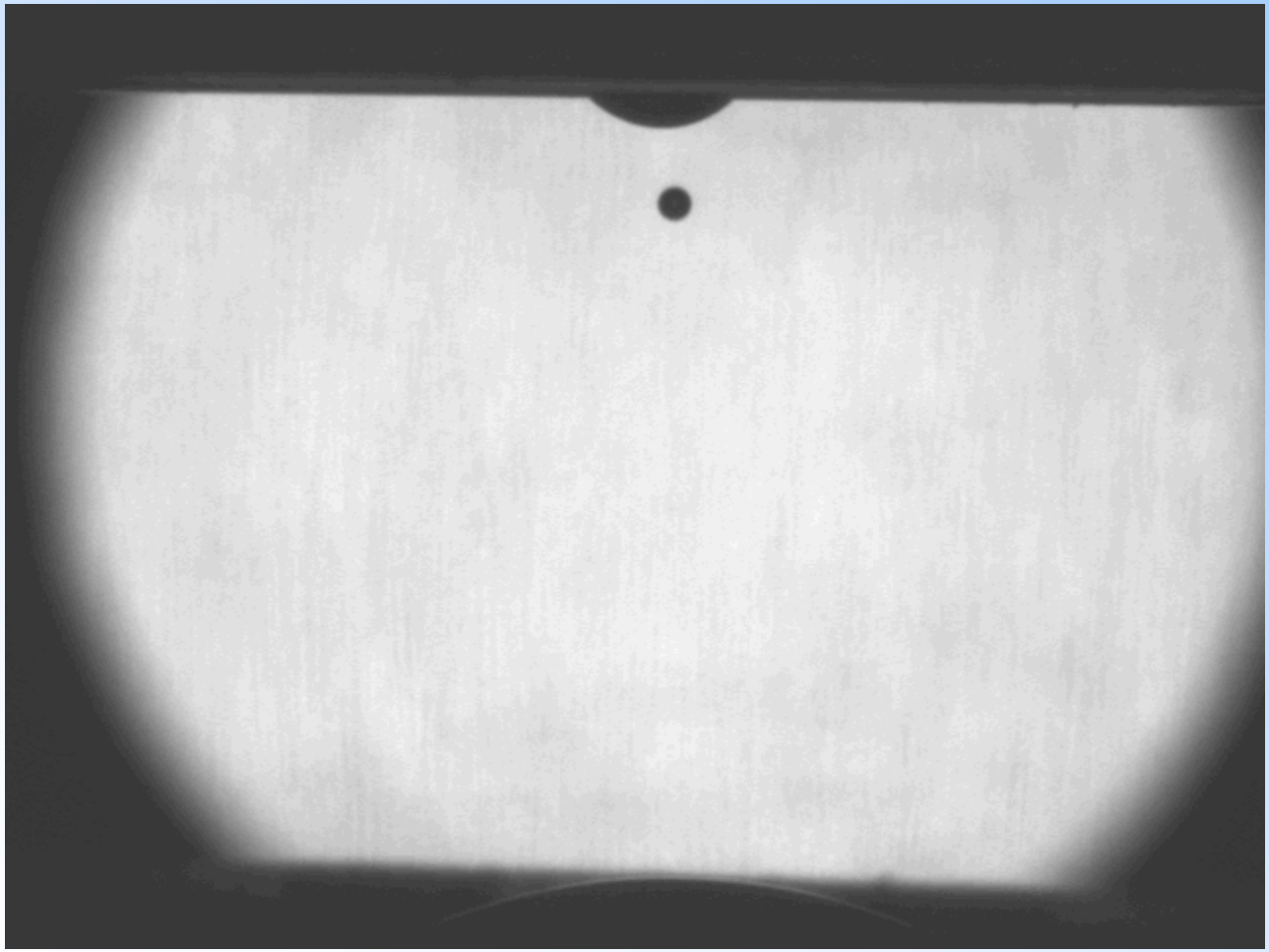
Interesting Phenomena



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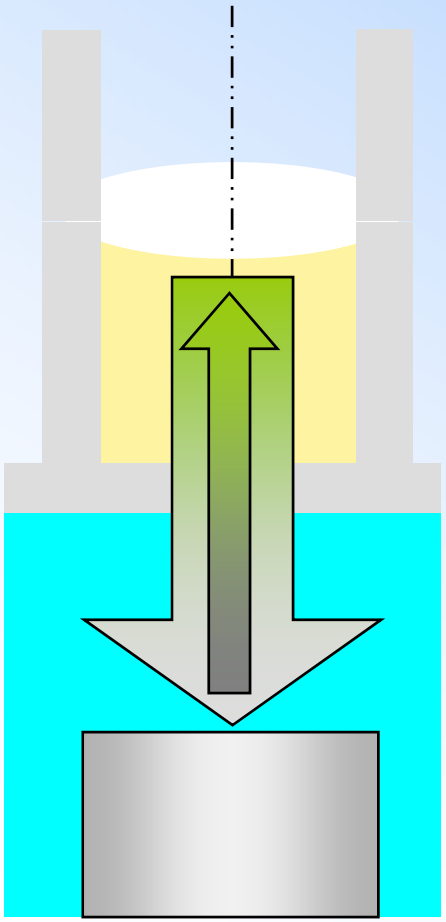
Fluid Detection



- **Acoustics enable “echo detection” of fluid**
- **Drop formation**
  - Delivery of energy to the focal spot
- **Echo detection**
  - Energy reflected back to the transducer

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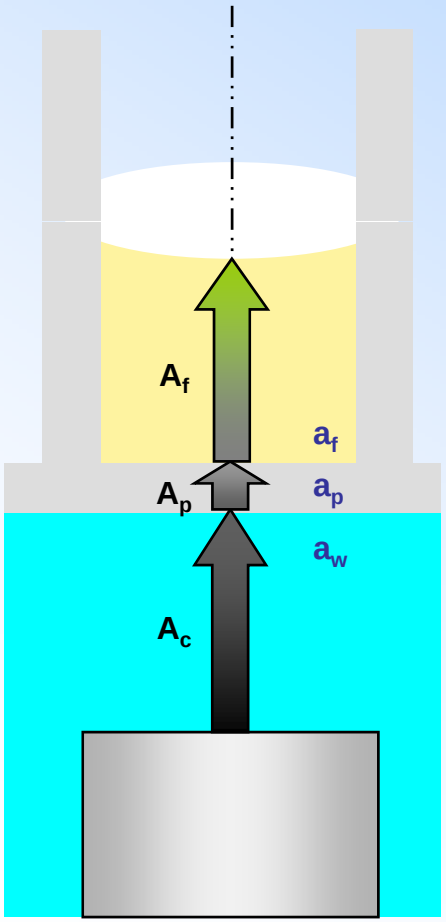
Acoustic Path: Attenuation



- **Acoustic energy is lost as the waves travel from the source to the focus**

$$A = A_f + A_p + A_c$$

A_f Attenuation through fluid
A_p Attenuation through plate
A_c Attenuation through coupler

a_f Fluid attenuation (dB/mm for frequency F)
a_p Plate attenuation (dB/mm for frequency F)
a_c Coupler attenuation (dB/mm for frequency F)

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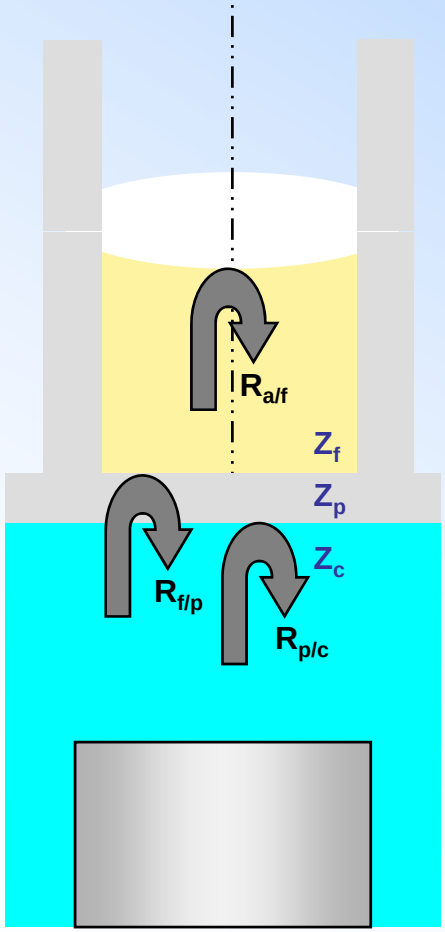
Acoustic Path: Reflections



- **Acoustic energy is reflected from interfaces where acoustic impedance of materials do not match**

$$R_{1/2} = \frac{(Z_1 - Z_2)^2}{(Z_1 + Z_2)^2}$$

$R_{a/f}$ Reflection from air/fluid interface
$R_{f/p}$ Reflection from fluid/plate interface
$R_{p/c}$ Reflection from plate/coupler interface

Z_f Fluid acoustic impedance
Z_p Plate acoustic impedance
Z_c Coupler acoustic impedance

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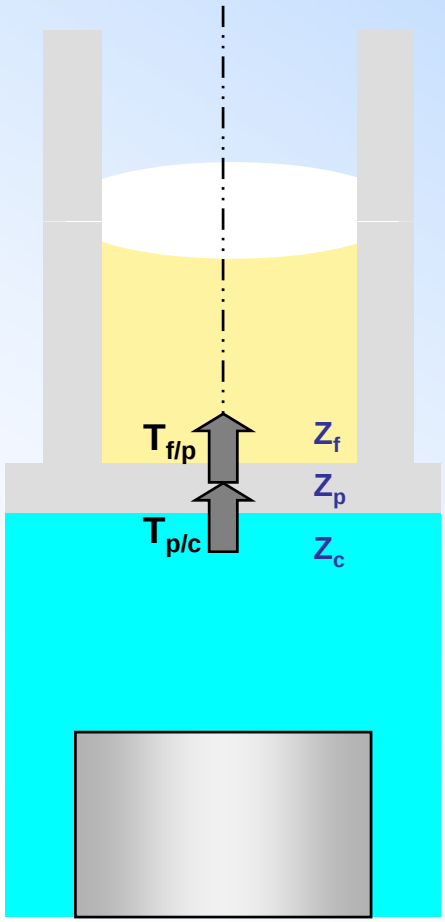
Acoustic Path: Transmission



- **Acoustic energy is transmitted through interfaces based on degree of acoustic impedance match**

$$T_{1/2} = \frac{4 Z_1 Z_2}{(Z_1 + Z_2)^2}$$

$T_{f/p}$ Reflection from fluid/plate interface
$T_{p/c}$ Reflection from plate/coupler interface

Z_f Fluid acoustic impedance
Z_p Plate acoustic impedance
Z_c Coupler acoustic impedance

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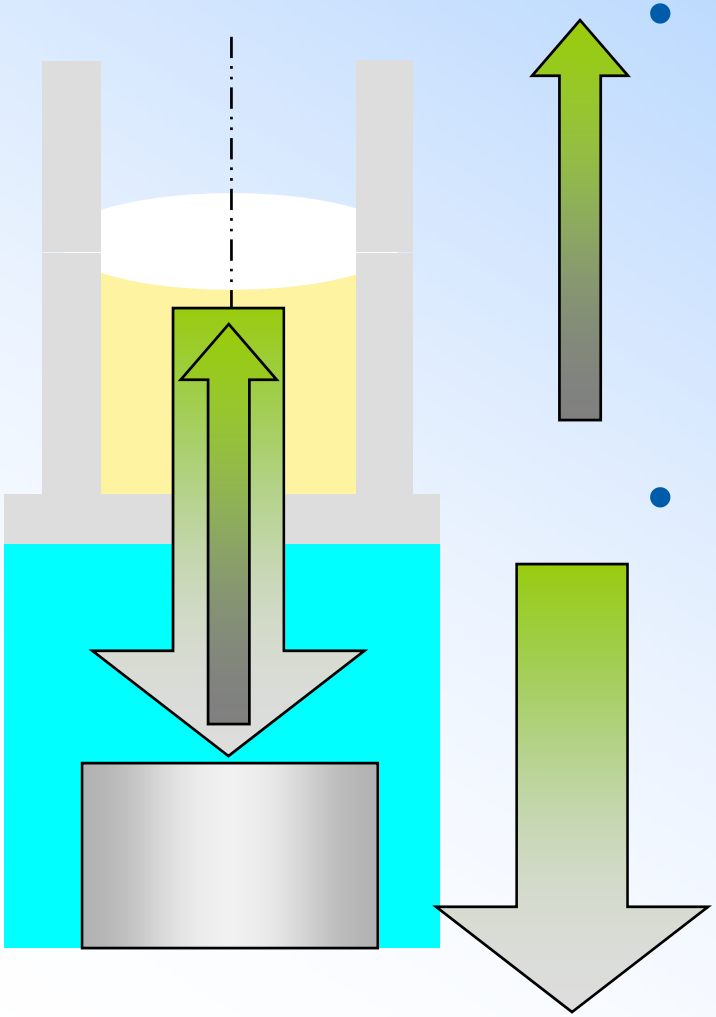
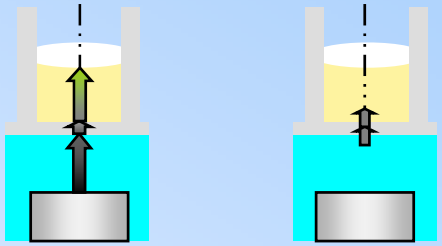
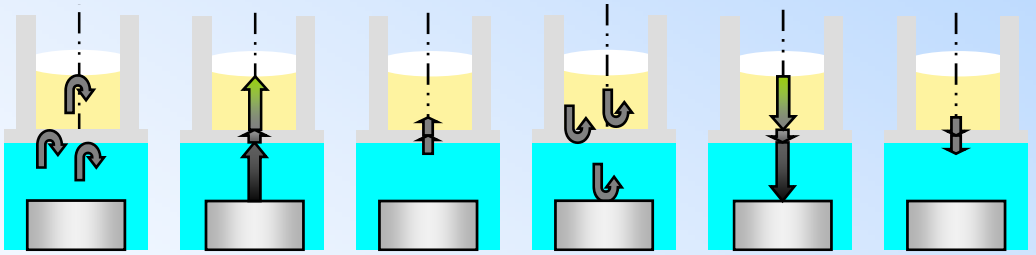
Acoustic Path



- Drop formation



- Detection



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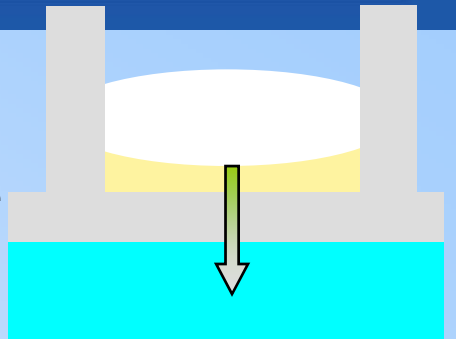
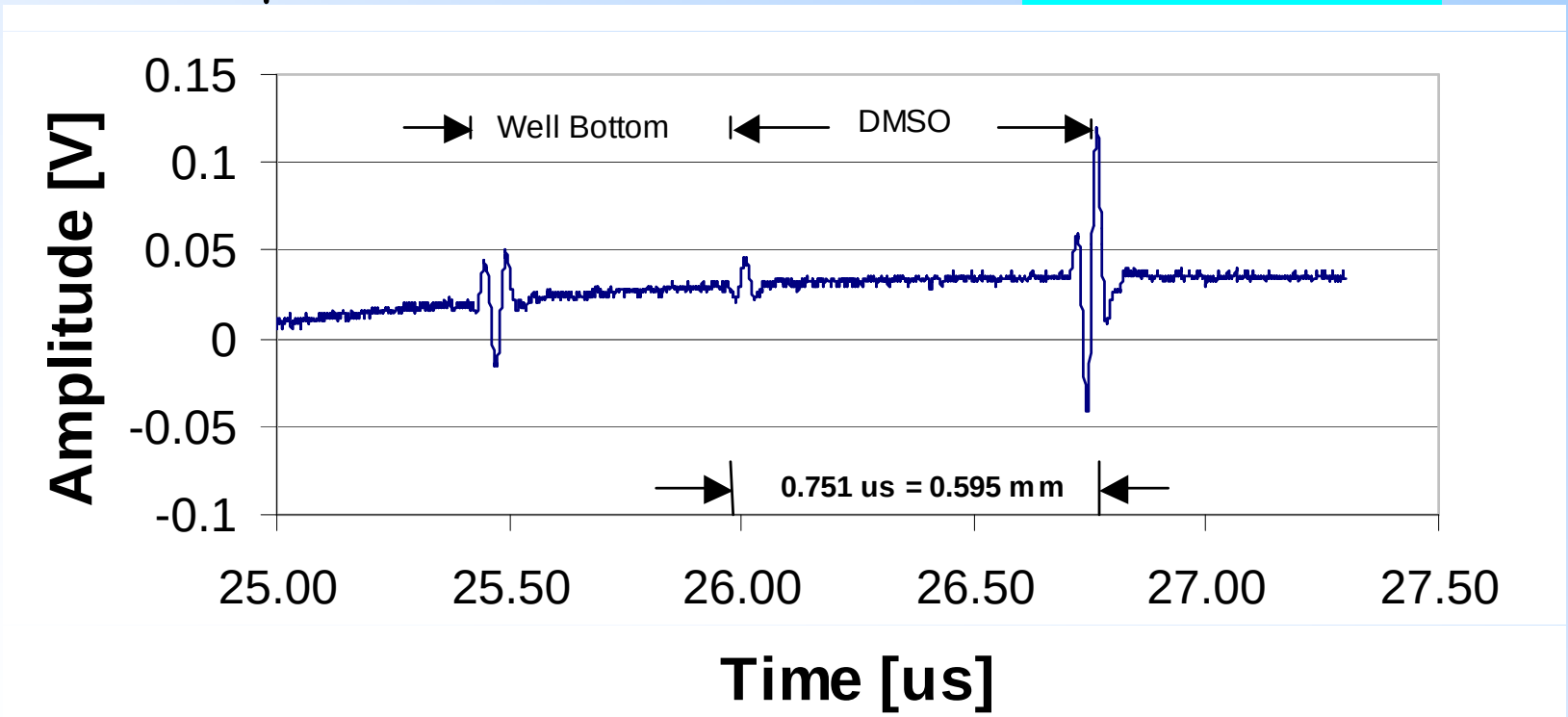
Fluid Depth Detection

- **Acoustic Echo Signal**
  - 384 well in polypropylene plate
  - 10 μL DMSO fill



Single point audit



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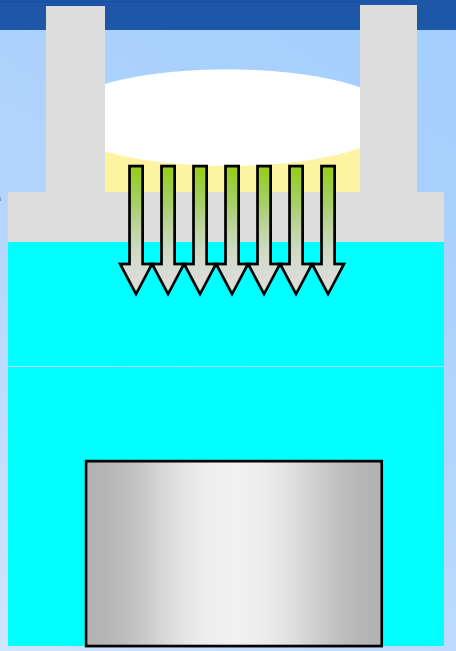
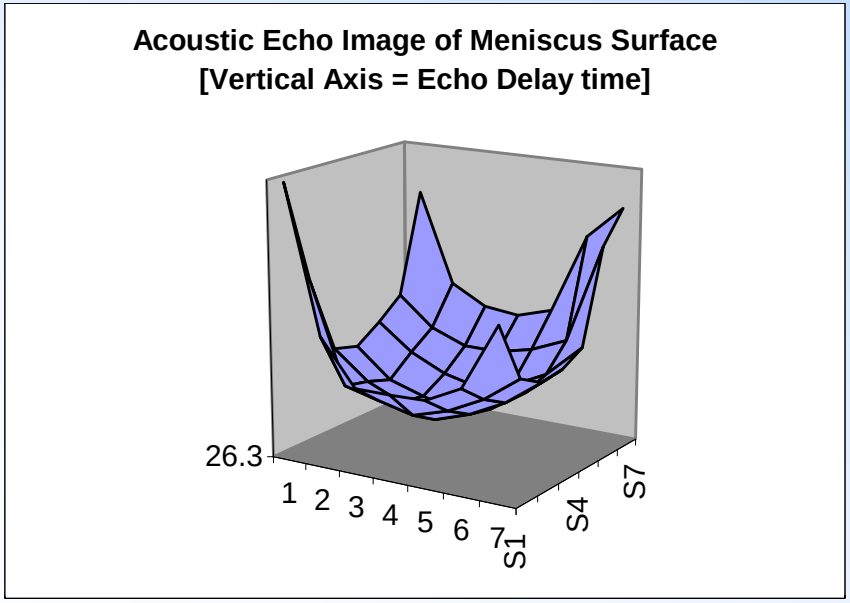
Meniscus Detection

- **Acoustic Echo Signal**
  - 384 well in polypropylene plate
  - 10 μL DMSO fill



Multiple point audit



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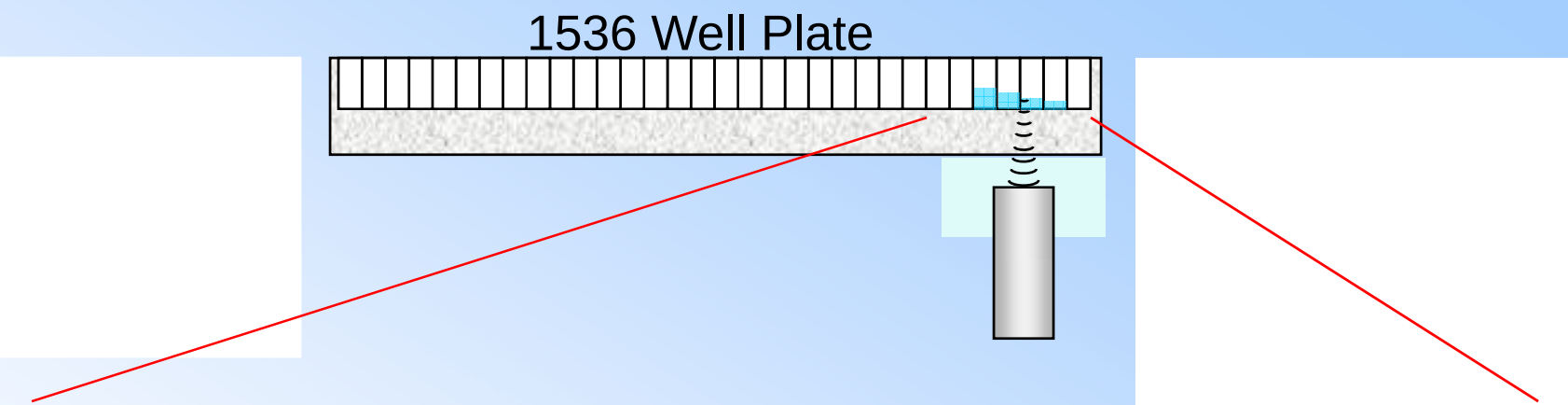
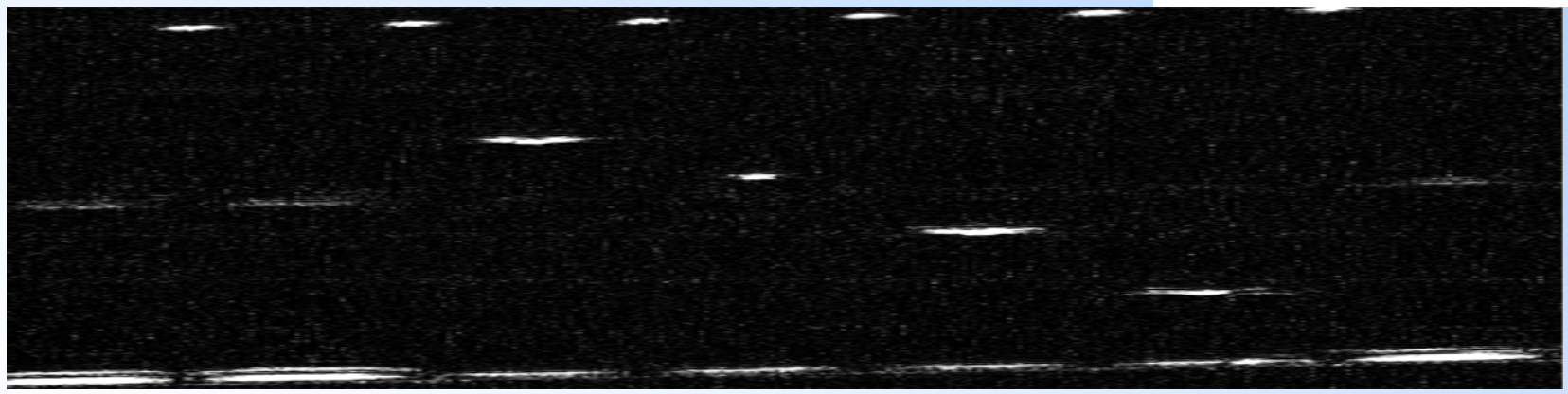
Well Plate Sonogram





M42 M43 M44 M45 M46 M47 M48

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eksigent

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