Microfluidic Chips for DNA Analysis

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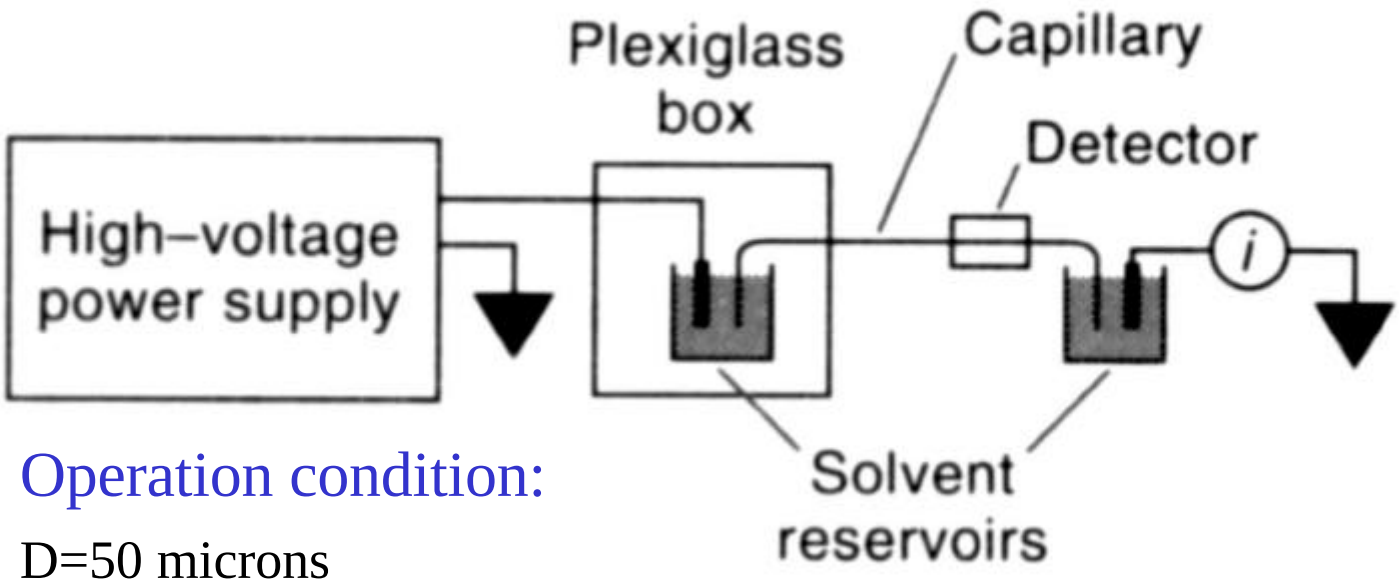
Outline

- **Introduction**
- **Design and Fabrication of μ-fluidic chips**
- **Embossing method for duplication of micro channels on PMMA substrates**
- **Chemical Analysis Applications**
  - ϕx174 DNA Maker separation
  - HCV

Conventional Capillary Electrophoresis



Operation condition:

D=50 microns

L=50 cm => time consuming

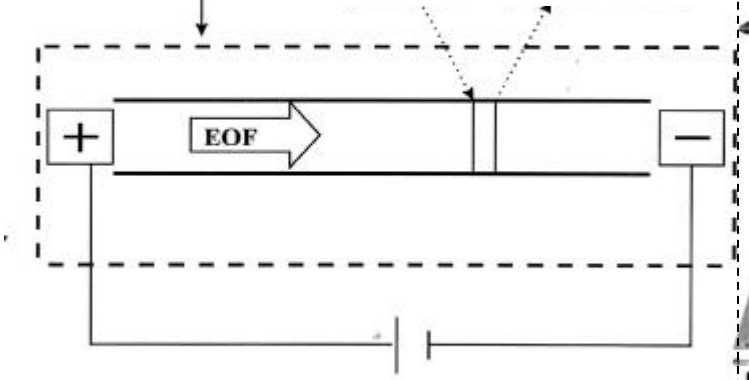
E=300 - 400 V/cm

I=10-50 μA

Capillary Electrophoresis Chip

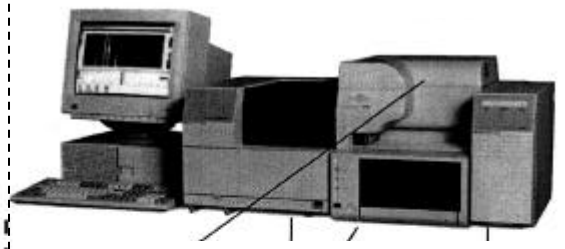
Micro channels

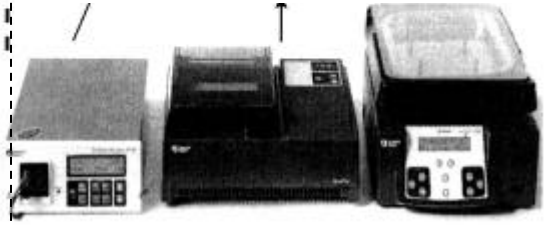
Optical detection

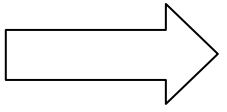


Power Supply

Commercial CE



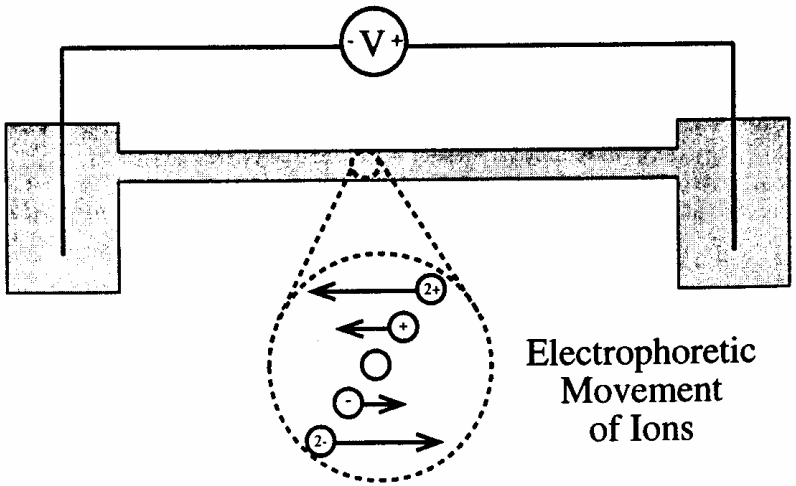




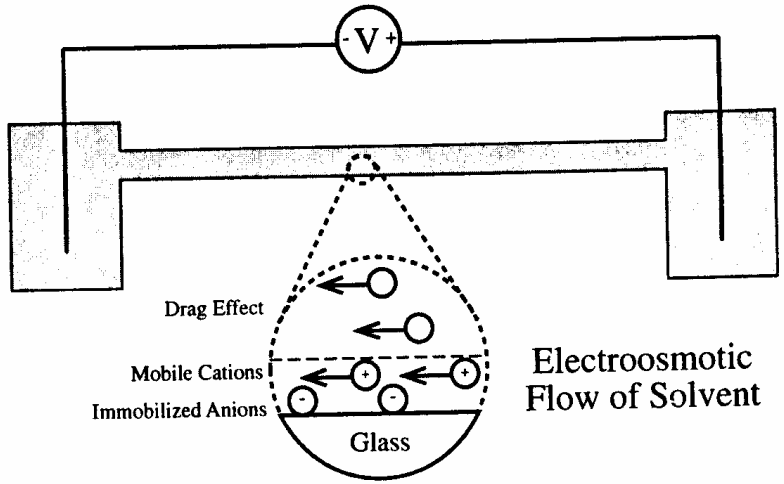
- faster
- cheaper
- lower voltage
- less sample
- integrated with sample handling system

Flow driven by electrophoretic/electroosmotic pumping

*Continuous presence of ions in a suitable solvent
*Flow driven by electric field



Conceptual illustration of electrophoresis. After Manz, et al. (1994).



Conceptual illustration of electroosmotic pumping. After Manz, et al. (1994).

Characteristics of PMMA

Advantages:

- simple extension to fabrication

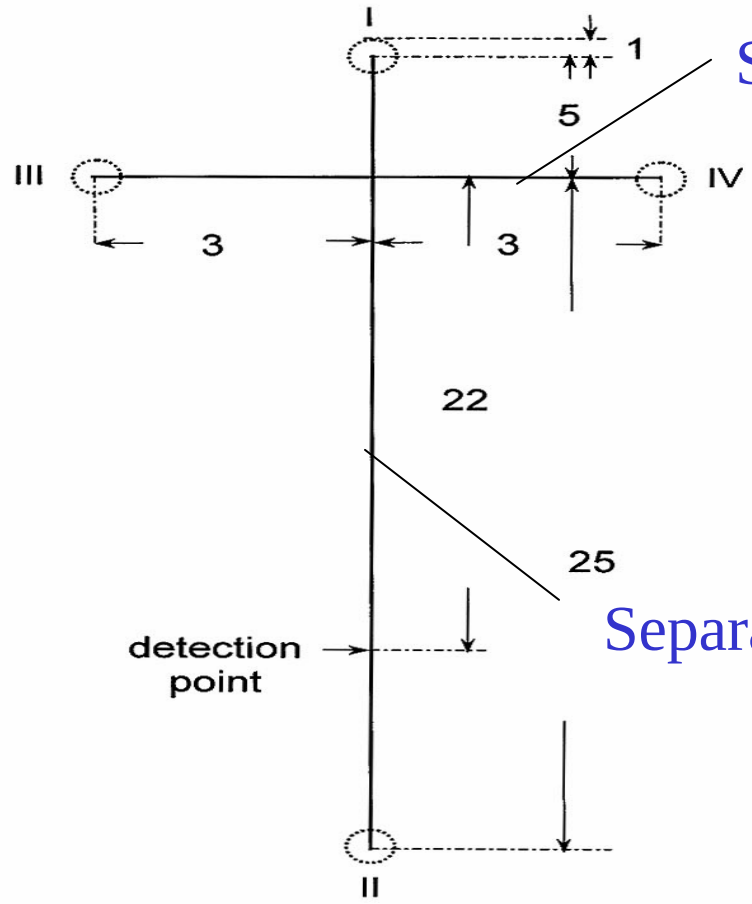
  e.g.. Thermal embossing, injection molding etc.
- low cost
- low adsorption for biomolecules (DNA, protein….)
- usable wavelength (visible region)

Disadvantages:

- Poor chemical resistance
- Poor thermal dissipation

Design of micro channels

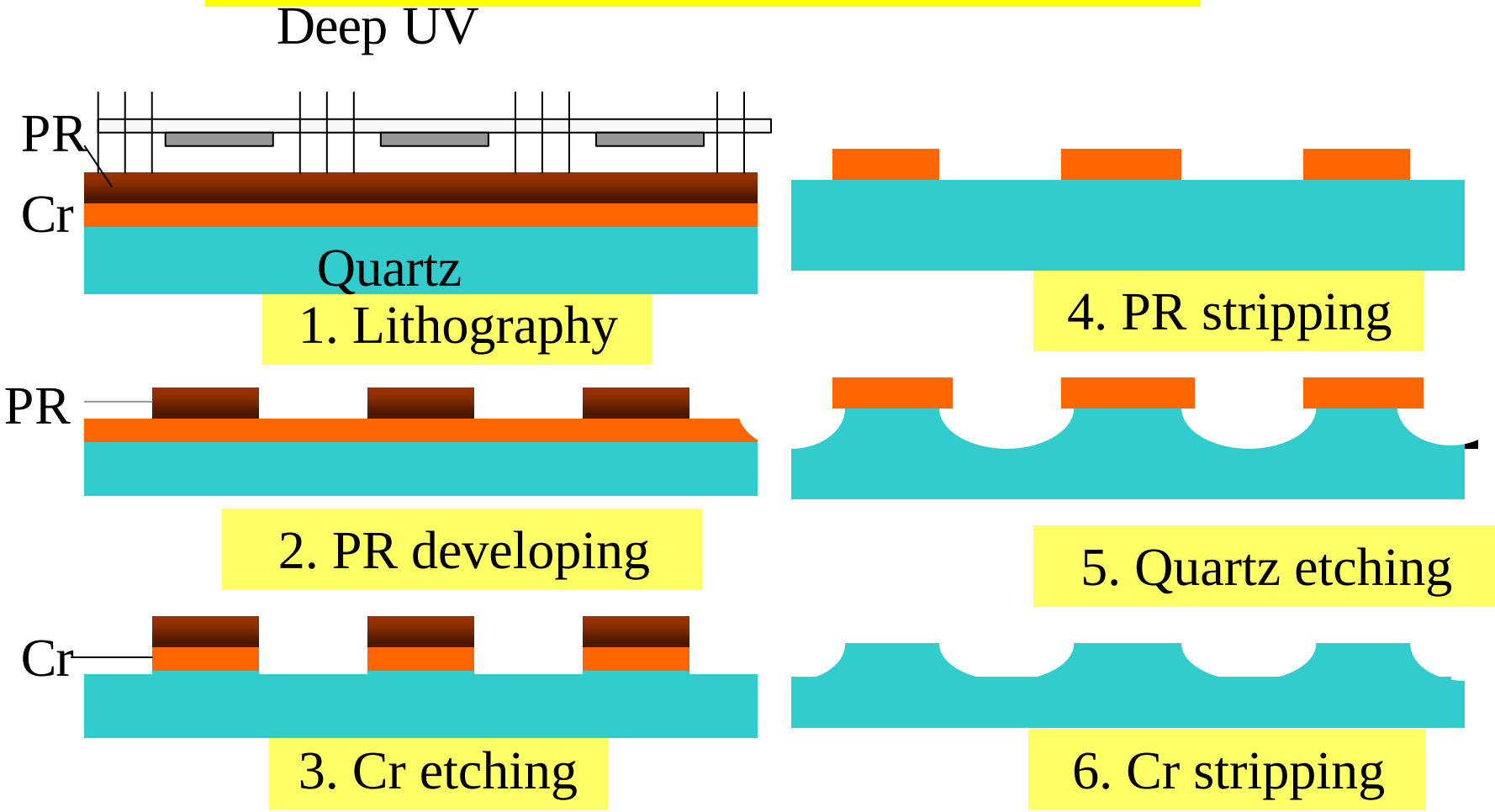
Unit : mm



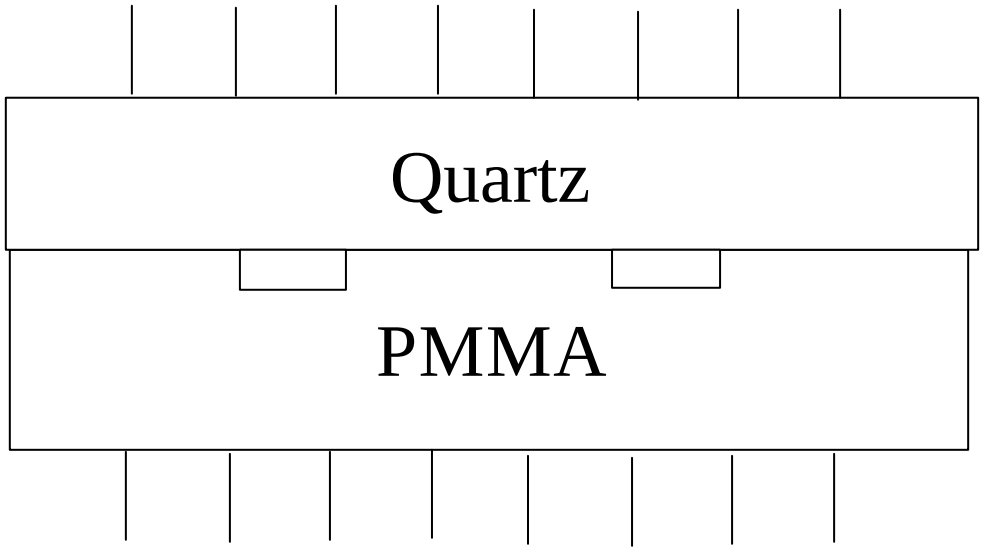
Sample channel

Separation channel

Fabrication Process



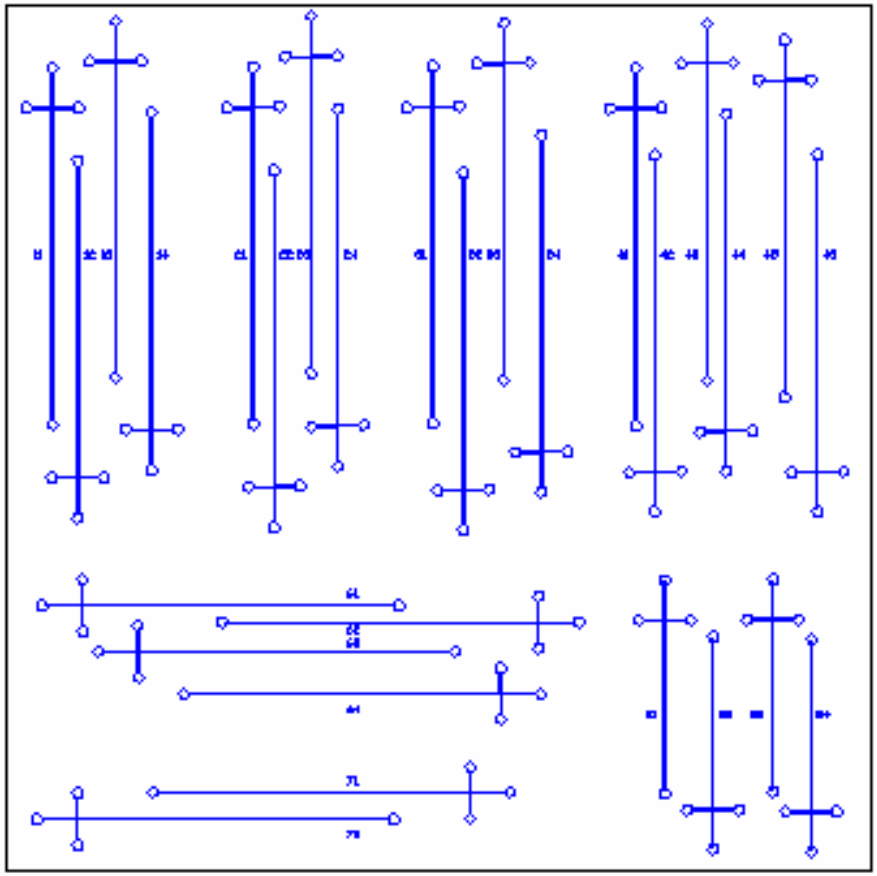
Embossing method for PMMA chips



- Transfer of inverse images on quartz template onto PMMA base plates
- Heat treatment + Compression
- good reproducibility for PMMA channel (RSD $\leqq$ 3%)

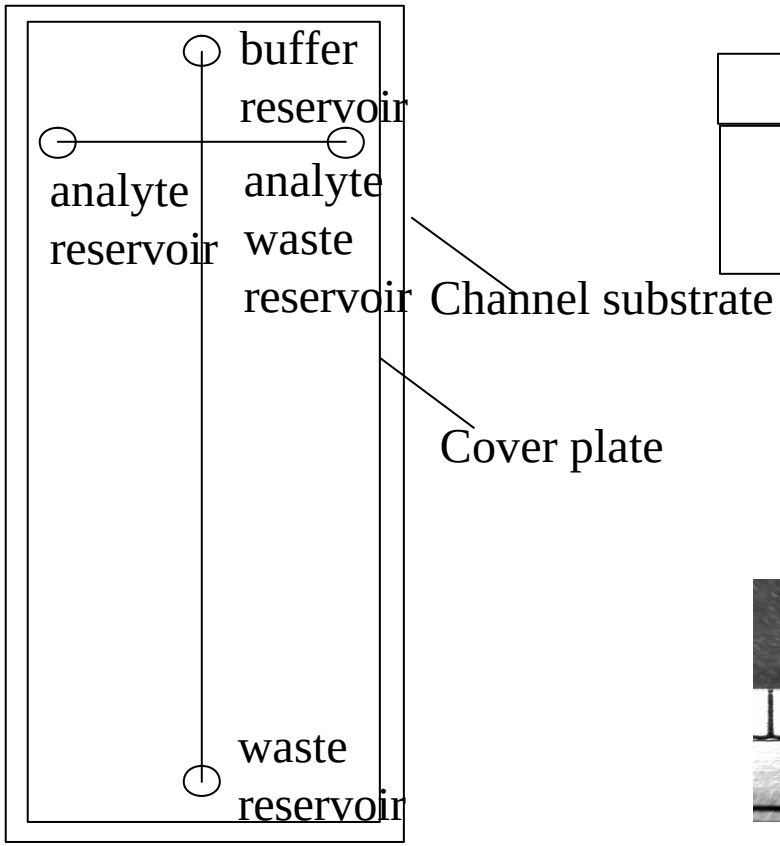
=> easy duplication of cheap and disposable plastic chips

Design of Quartz Templates

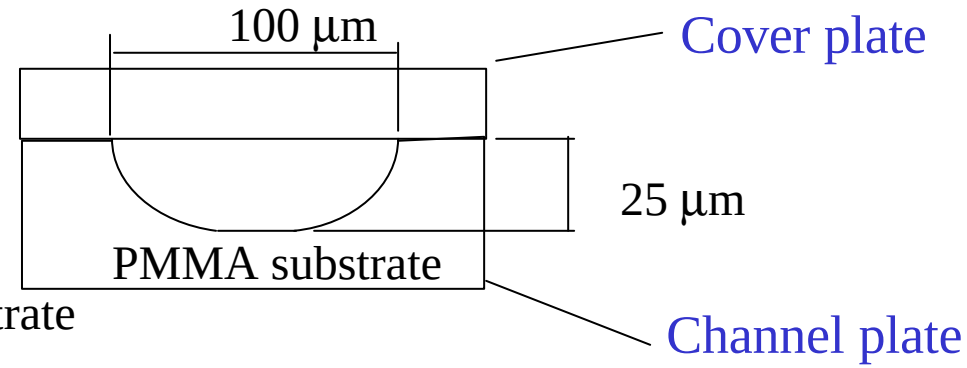


Microfabricated Capillary Electrophoresis Chips

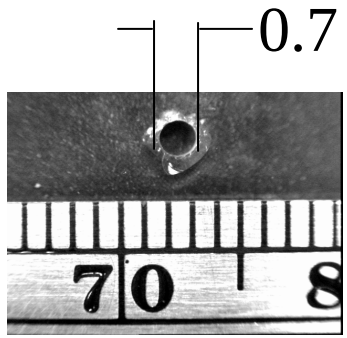
Top view



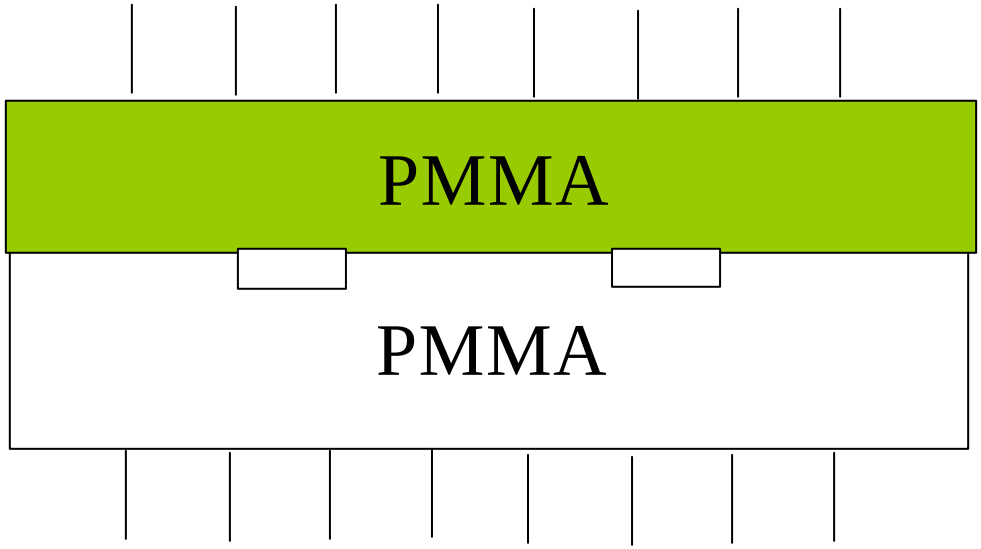
Cross section view



Through hole



Bonding for PMMA chips

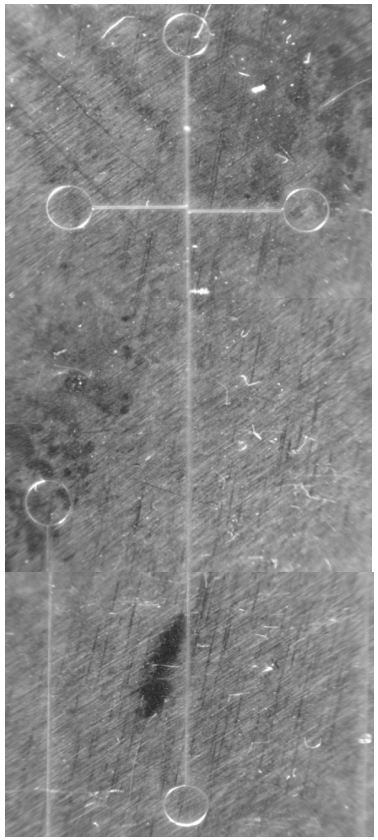


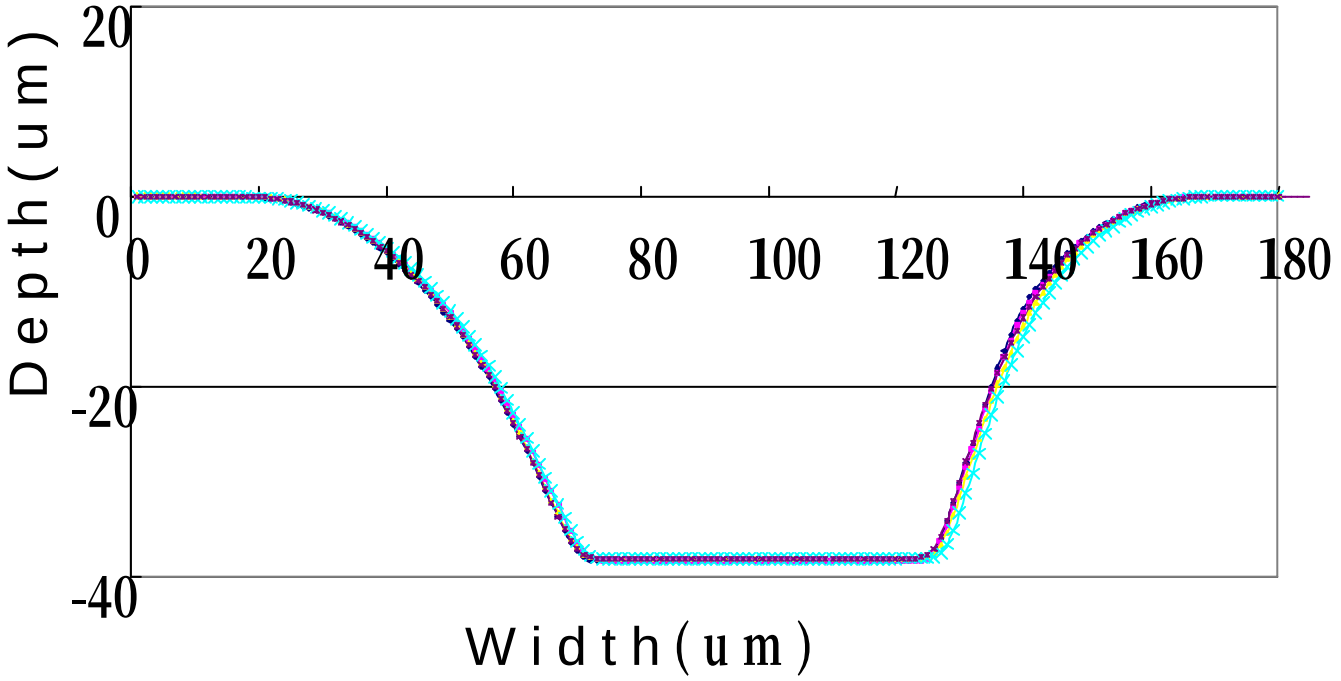
- PMMA bonding by heating plastics above transition temperature.

- Temperature and pressure control are critical for bonding quality.

=> good sealing is observed

Surface profile



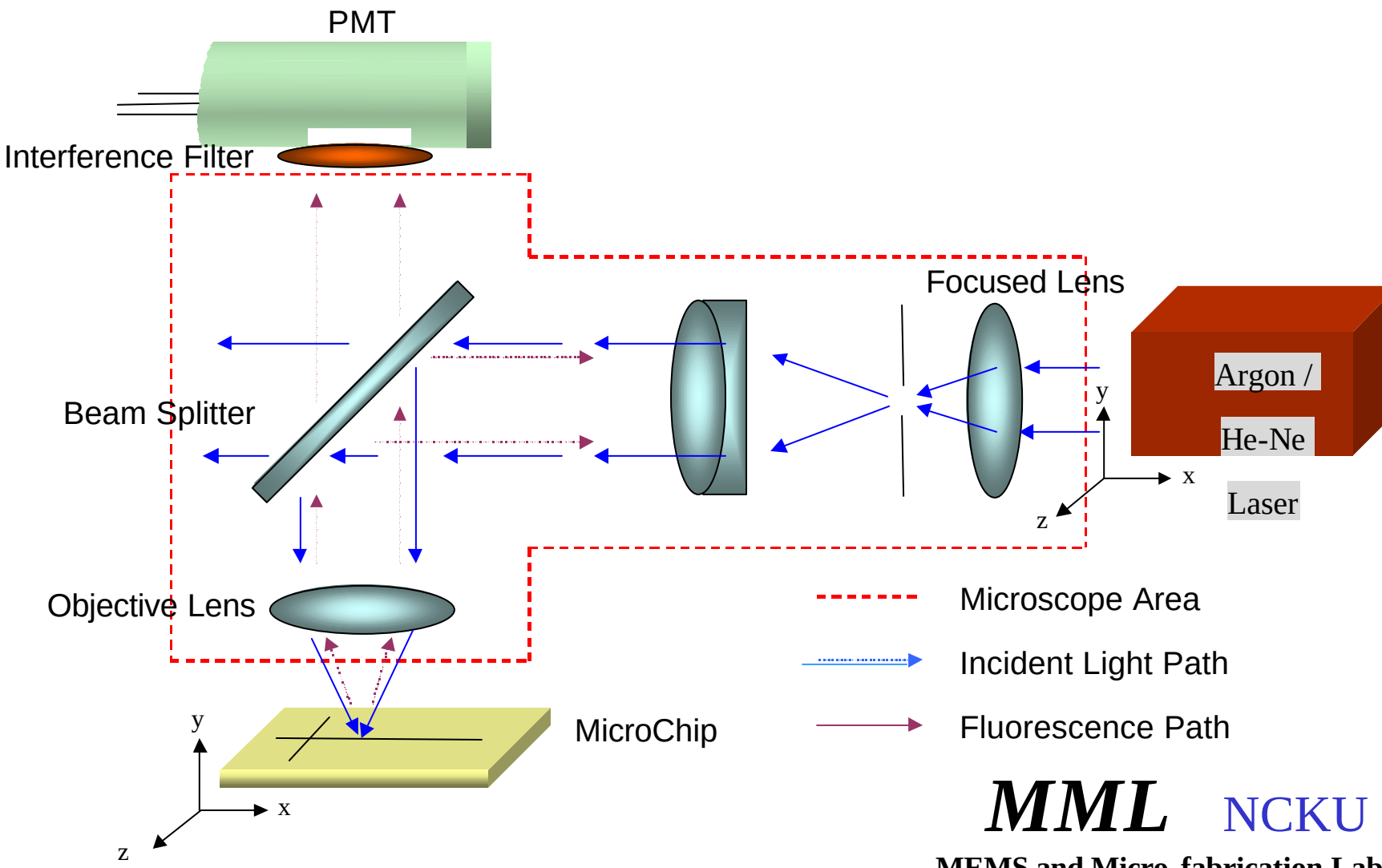


Micro Channels on PMMA chips

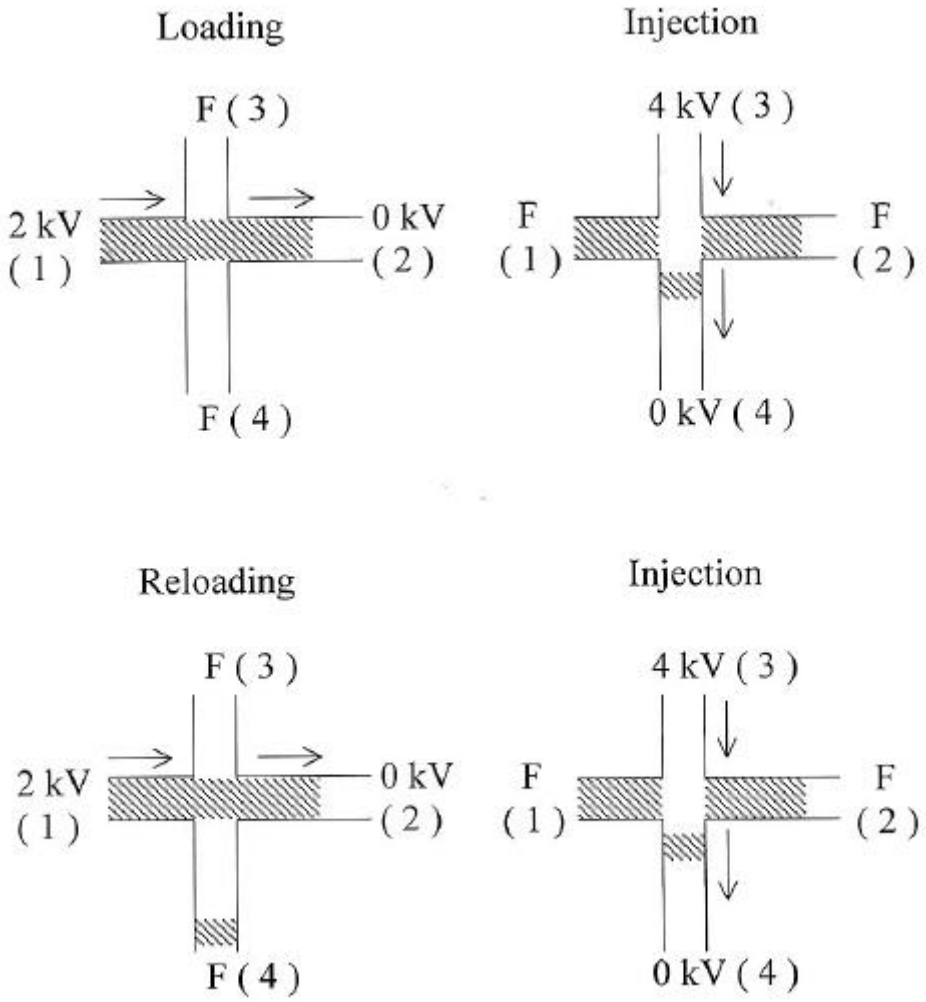
Channel #	Depth (μm)	Width (μm)
1	25.0	100.0
2	25.2	102.0
3	24.5	103.0
4	24.0	101.0
5	25.3	104.0
6	25.2	103.0
7	27.0	108.0
8	26.0	101.0
9	24.2	103.0
10	24.8	104.0
Average	25.12	102.9
SD	0.879141	2.233582
RSD	0.0350	0.0217

=>good reproducibility for PMMA channel (RSD $\leqq$ 1 %)

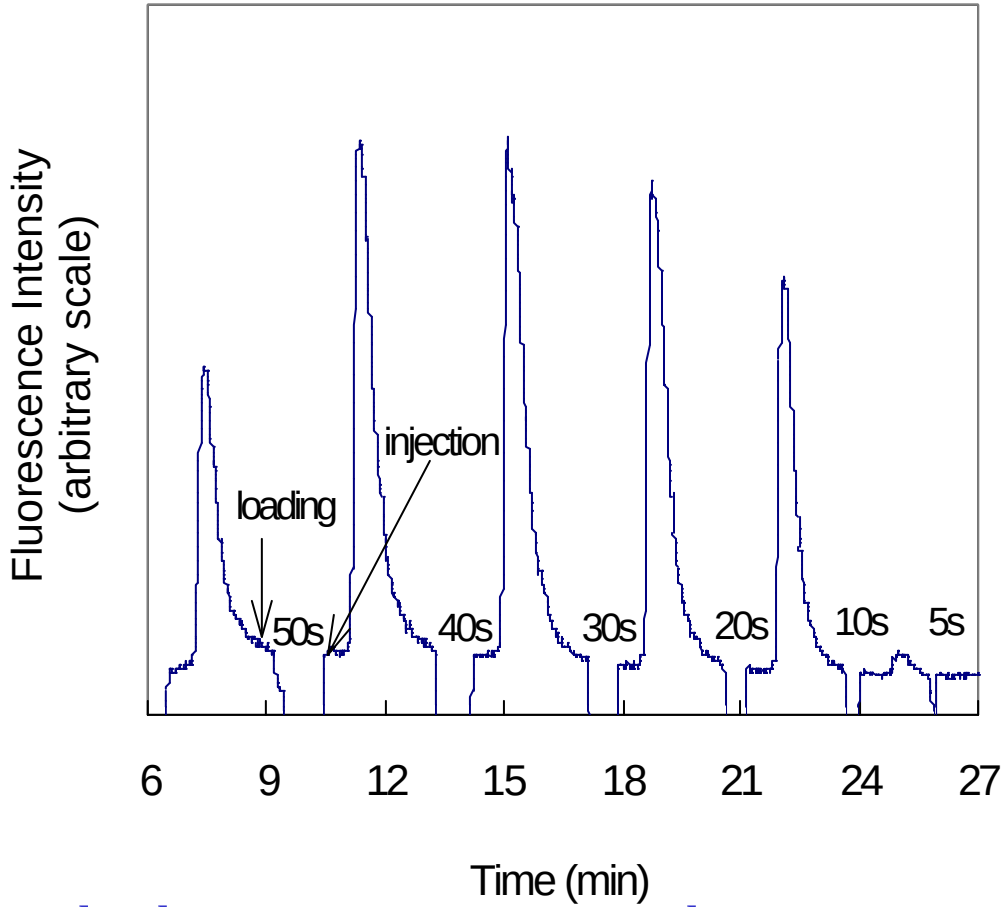
Schematic Diagram of Optical Detection System



Floating mode of sample injection and separation

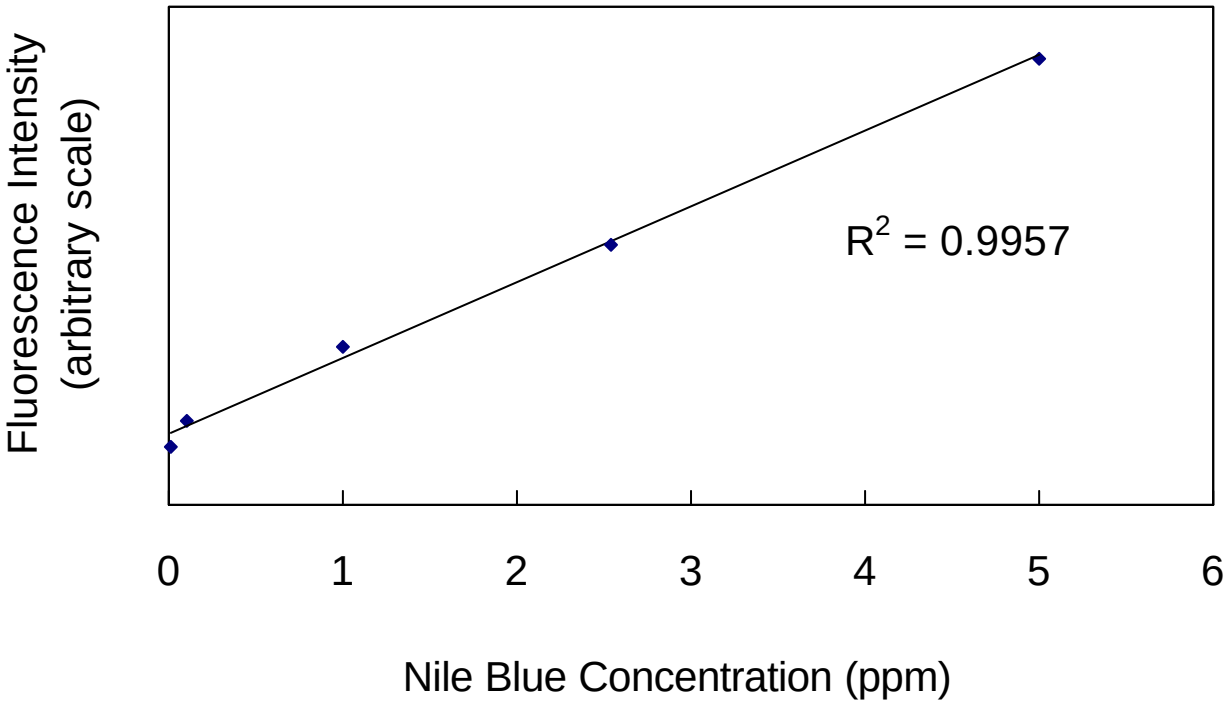


Consecutive injections of Nile blue (100 ppm) at various loading time

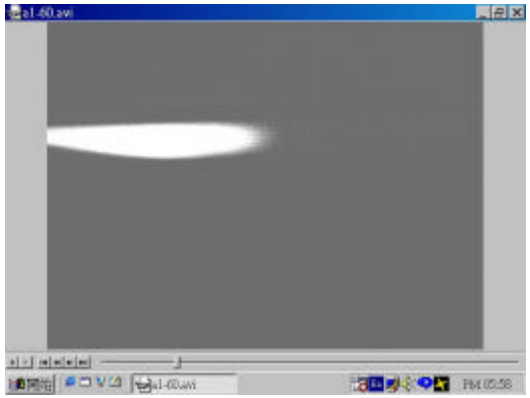


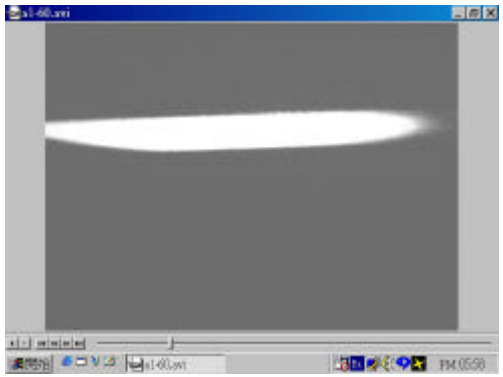
- optimum loading time: 30 ~ 50 seconds
- migration time: 0.63 min.
- excellent signal to noise ratio

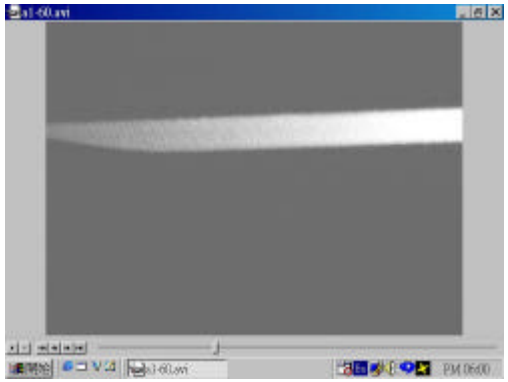
Calibration Curve of Nile Blue



Migration of Nile Blue inside a Micro Channel





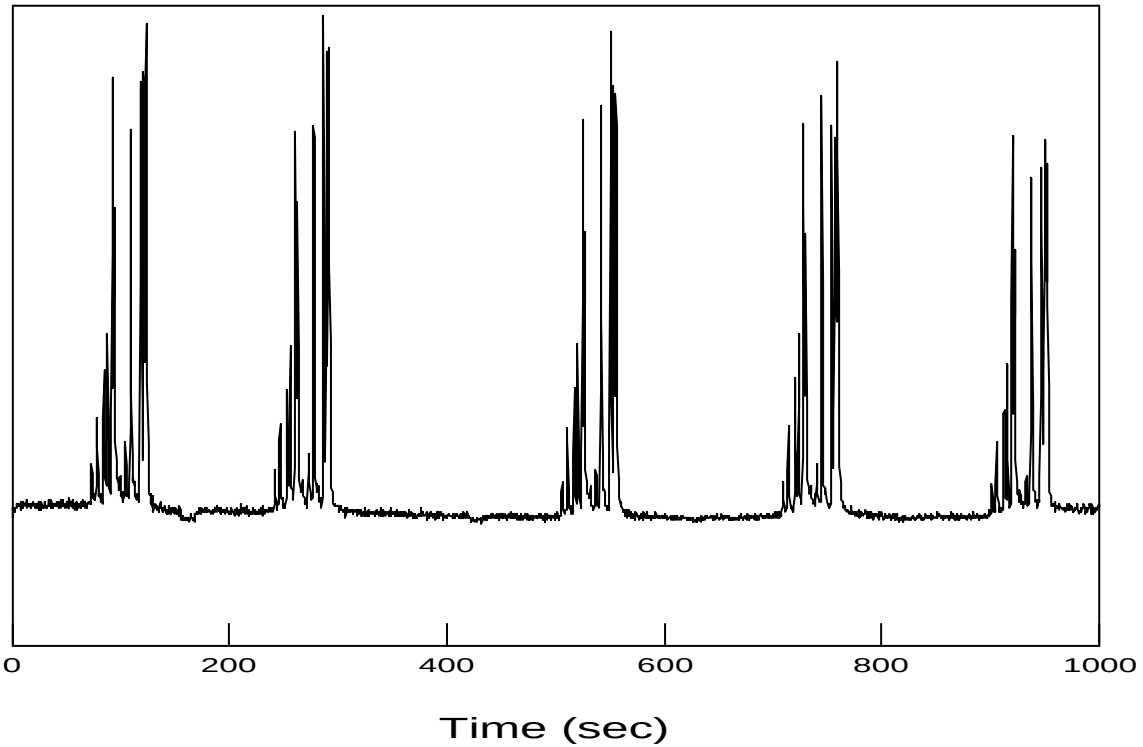


MML NCKU

MEMS and Micro-fabrication Lab

Dr. Gwo-Bin Lee

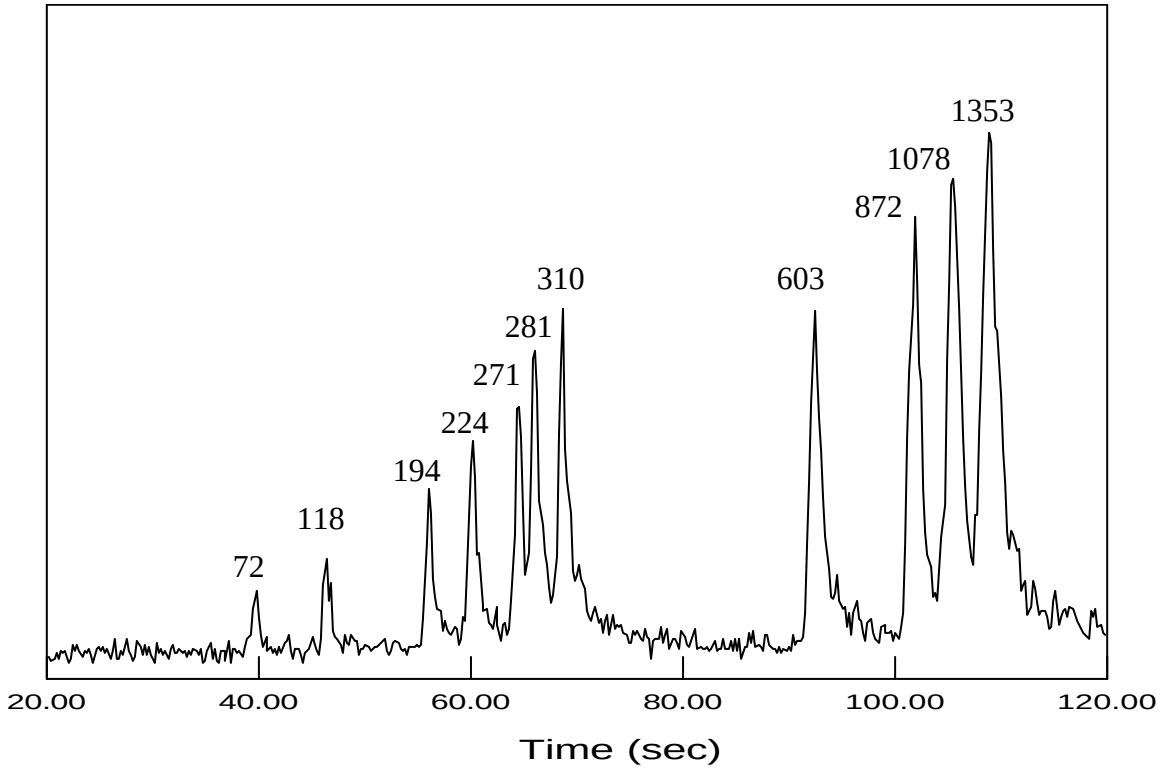
Electropherogram of Consecutive Injection - fx174 DNA Maker



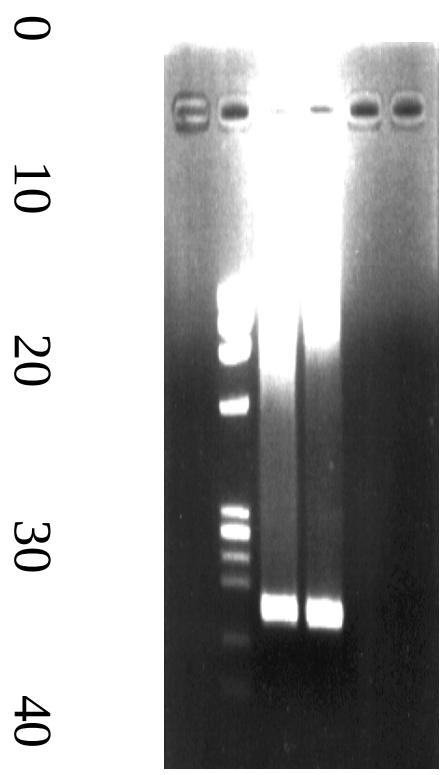
Relative standard deviation
< 0.4 % for migration
< 8 % for peak area

Time (min)

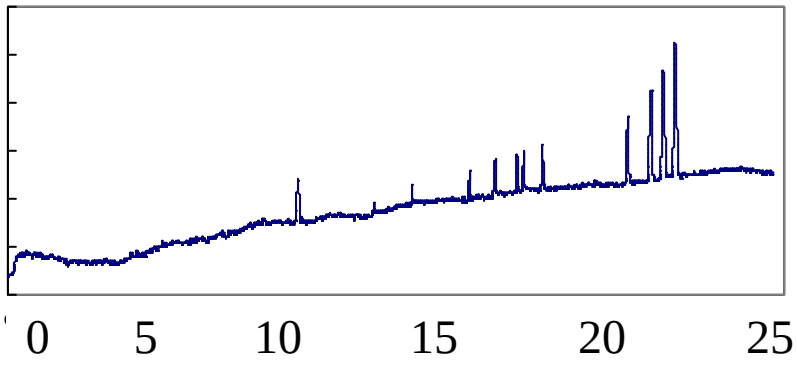
Separations of *f*x-174-RF DNA HaeIII



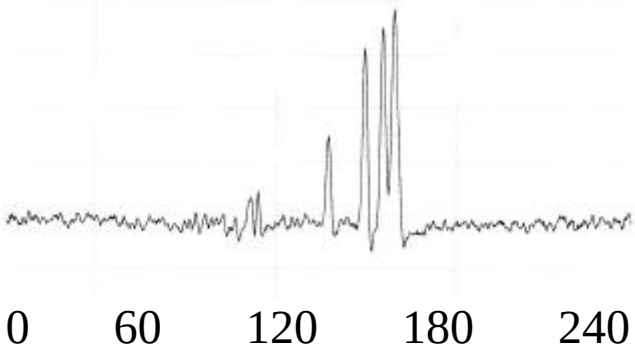
Electropherogram of fx174 DNA Maker for different electrophoresis methods



slab gel electrophoresis



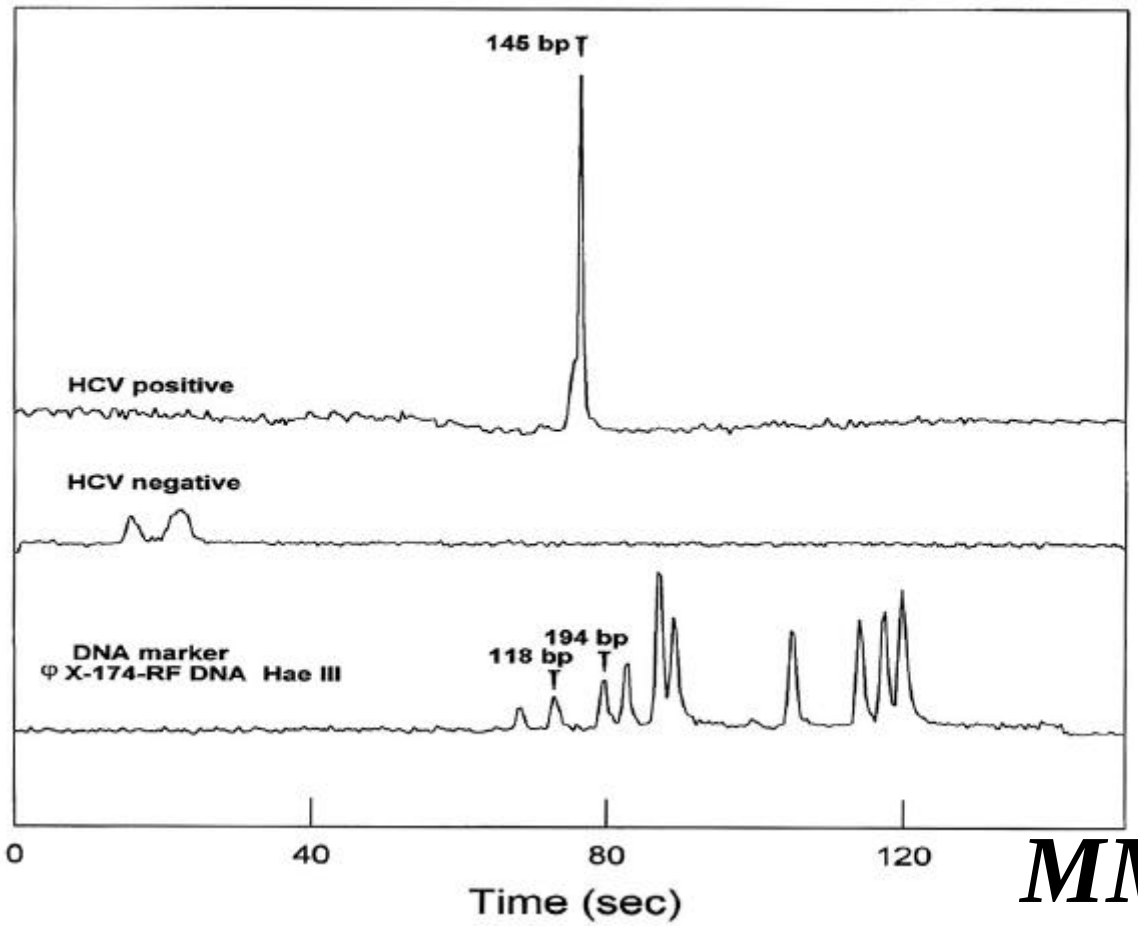
capillary electrophoresis



***chip* electrophoresis**

Clinical Diagnostics of Hepatitis C Virus

Microchip electrophorograms of the amplified HCV products



Conclusions

- Micro CE chips show potential for high-throughput chemical analysis
- Embossing duplication of PMMA chips is feasible
- low-cost, repeatable, disposable devices
- simple operation

ϕx-174 and HCV separation condition

matrix : 1% HPMC

buffer : 100 mM TBE pH=8.2

Separation Voltage : 1.0 KV = 333 V/cm

Light source : He-Neon laser 632.8nm

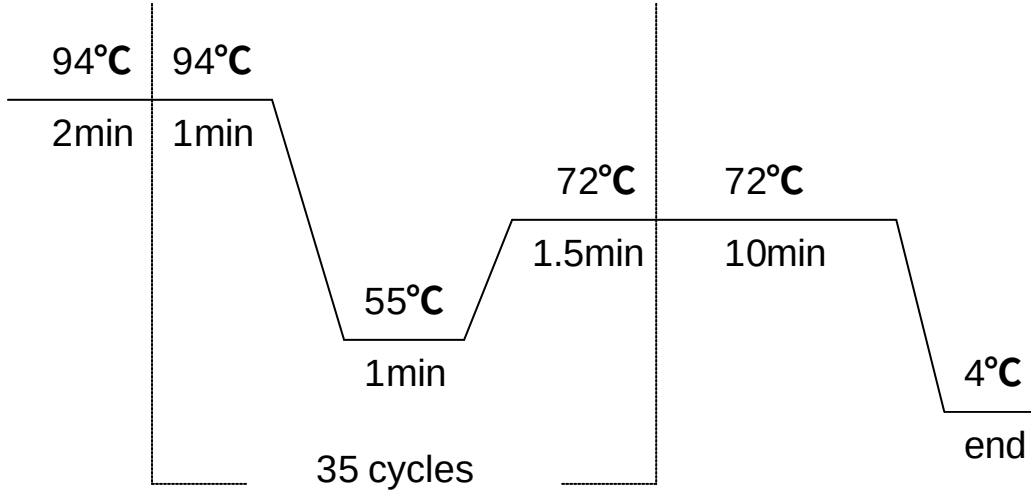
Dye: Intercalating Dye Topro 3

exciting wavelength : 642 nm

emmission wavelength : 660 nm

PCR Protocols of HCV

cDNA	10.0 μl
5mM dNTP	2.0 μl
10×PCR buffer	5.0 μl
20 μM 8RN	1.25 μl
20 μM 8LN	1.25 μl
Taq	0.5 μl
H_2O	30.0 μl
	50.0 μl



Clinical Diagnostics of Hepatitis C Virus

Nested PCR of HCV