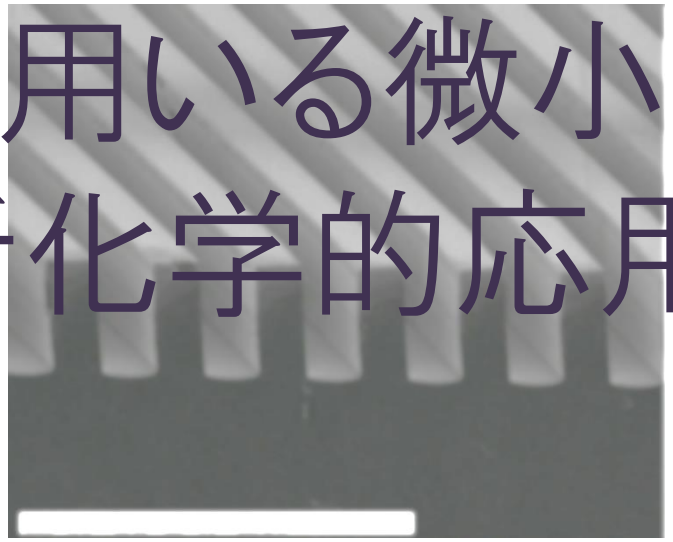
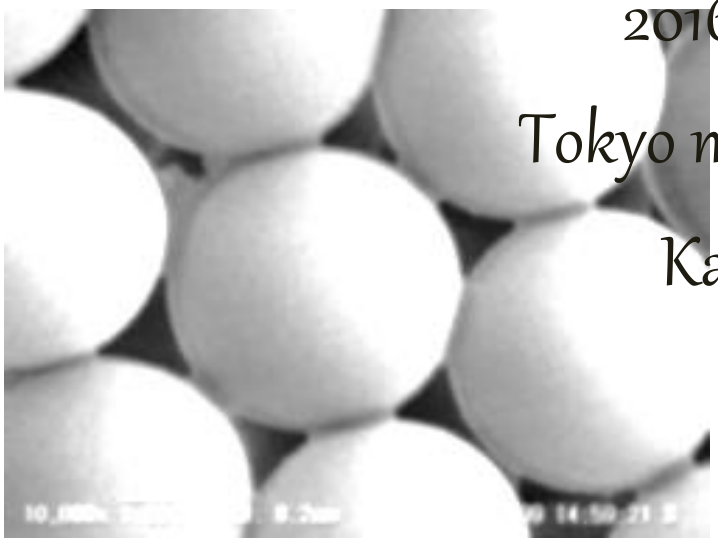
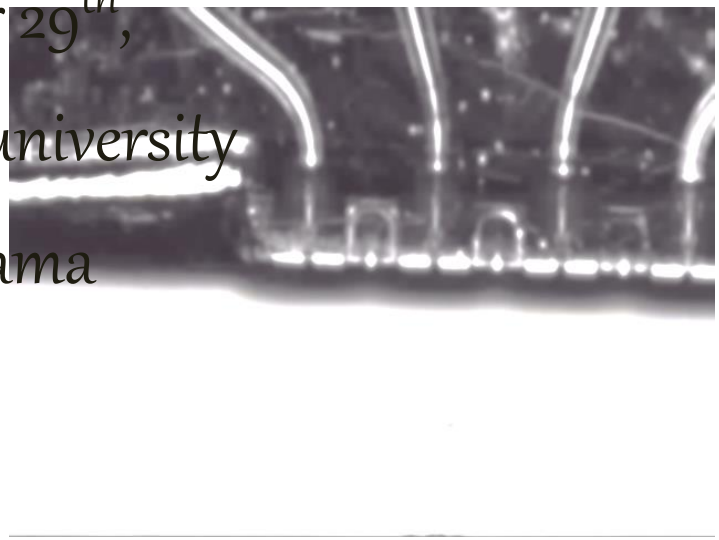
インクジェットを用いる微小液滴生成と分析化学的応用







2016　September 29th,

Tokyo metropolitan university

Katsumi Uchiyama

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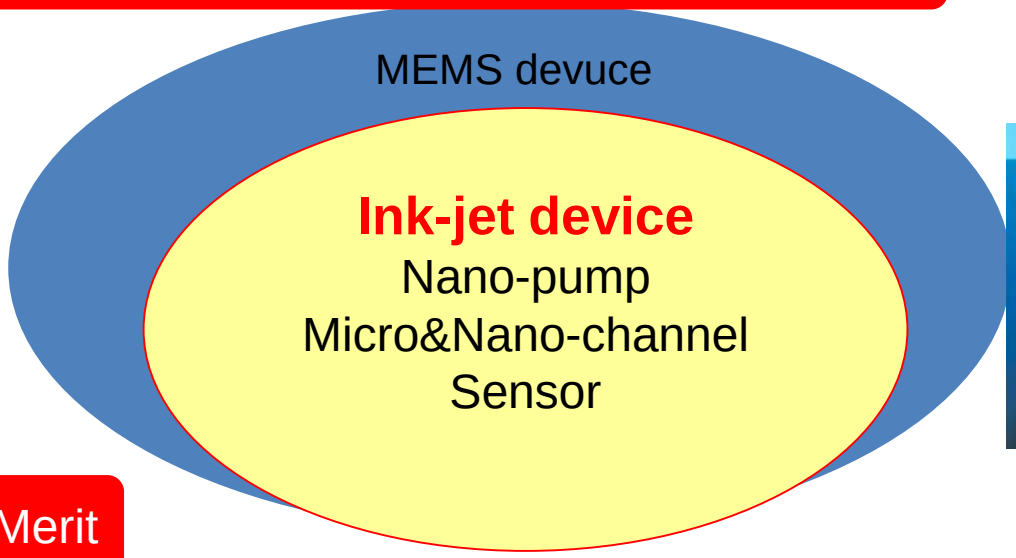
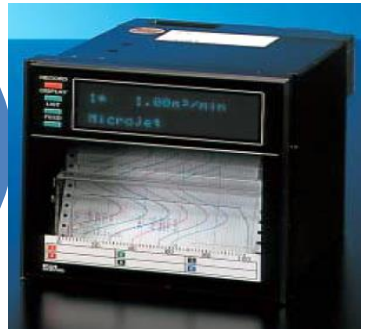
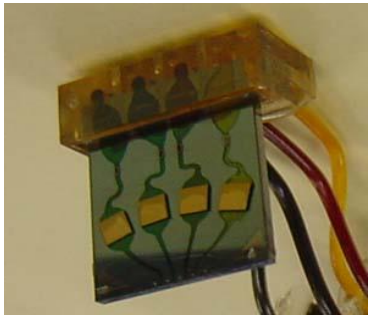
Ink jet micro-chip: overview

Micro fabrication technology







Industrial ink-jet recorder (left) and ink-jet head (right) (Fuji electronic systems Co. Ltd)

Merit

IJM ejects pico-liter amount of liquid samples at very high temporal and positional resolution with very high reproducibility.

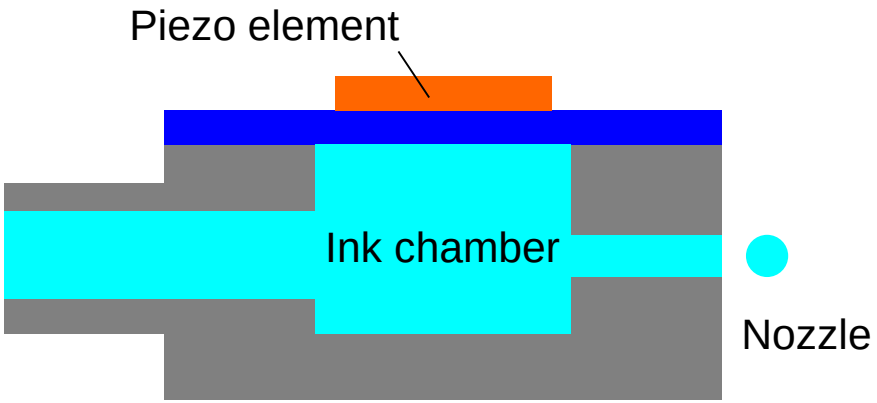
Dispensing Pico~Nano liter amount of liquid samples for surface reaction system on Analytical chemistry

Spotting technology for DNA micro array, nano-particles

Ink jet: PZT & BUBBULE JET

We used this device.

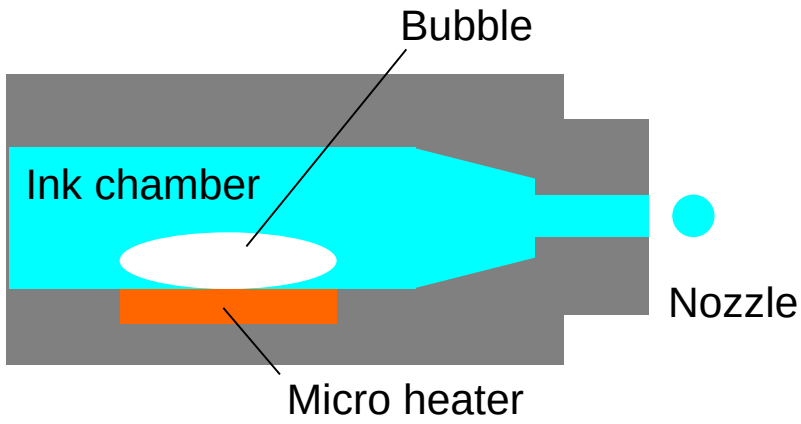
Piezo electric type



Pulse voltage (~100V, 50~100 micro-second) is applied to piezo element. Piezo element distorted to push out the liquid from nozzle.

- Generate no heat : fragile sample (Antibody, Antigen, enzymes etc)
- Very high reproducibility

Thermal ink-jet type

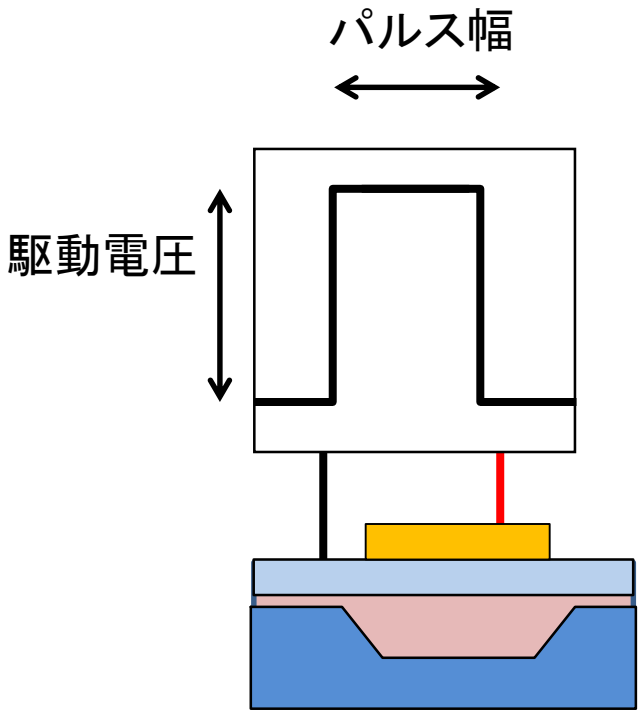


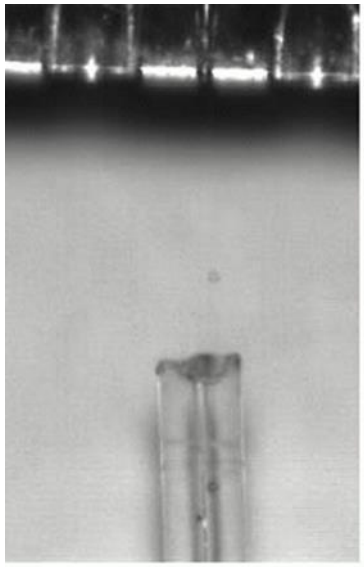
Micro heater heats ink at the chamber to make micro bubble. The bubble compress the chamber to eject the ink from nozzle.

駆動波形の最適化

再現性の良い試料導入

液滴の速度
直進性



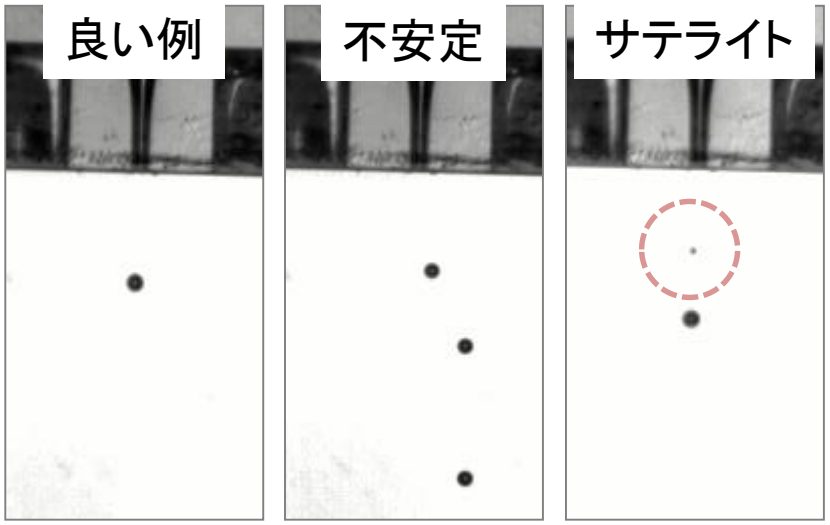


駆動波形：矩形波

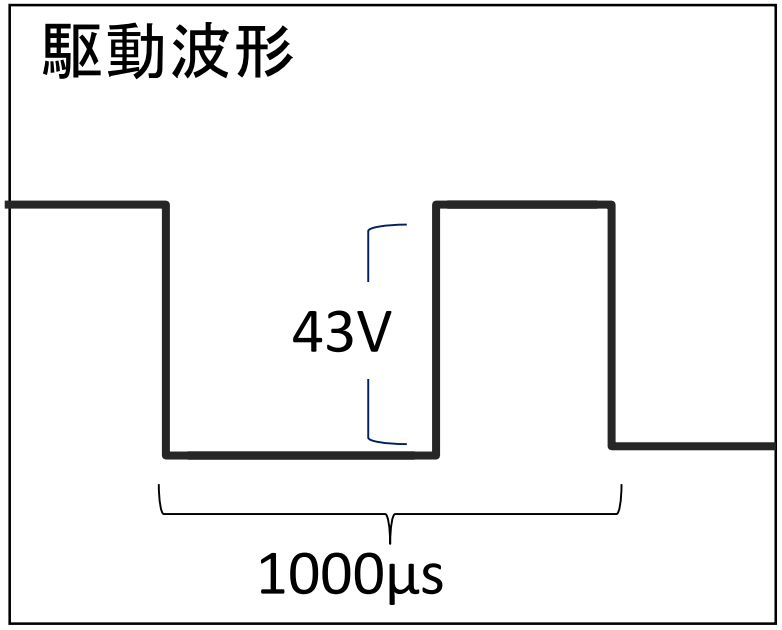
液滴の吐出速度は電圧に依存

駆動波形の最適化

再現性の良い吐出には
- 吐出が安定している
- サテライトが形成されない



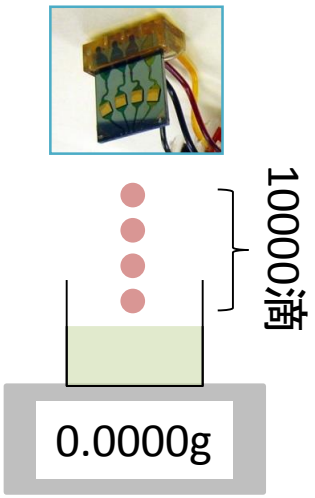
最適化した波形

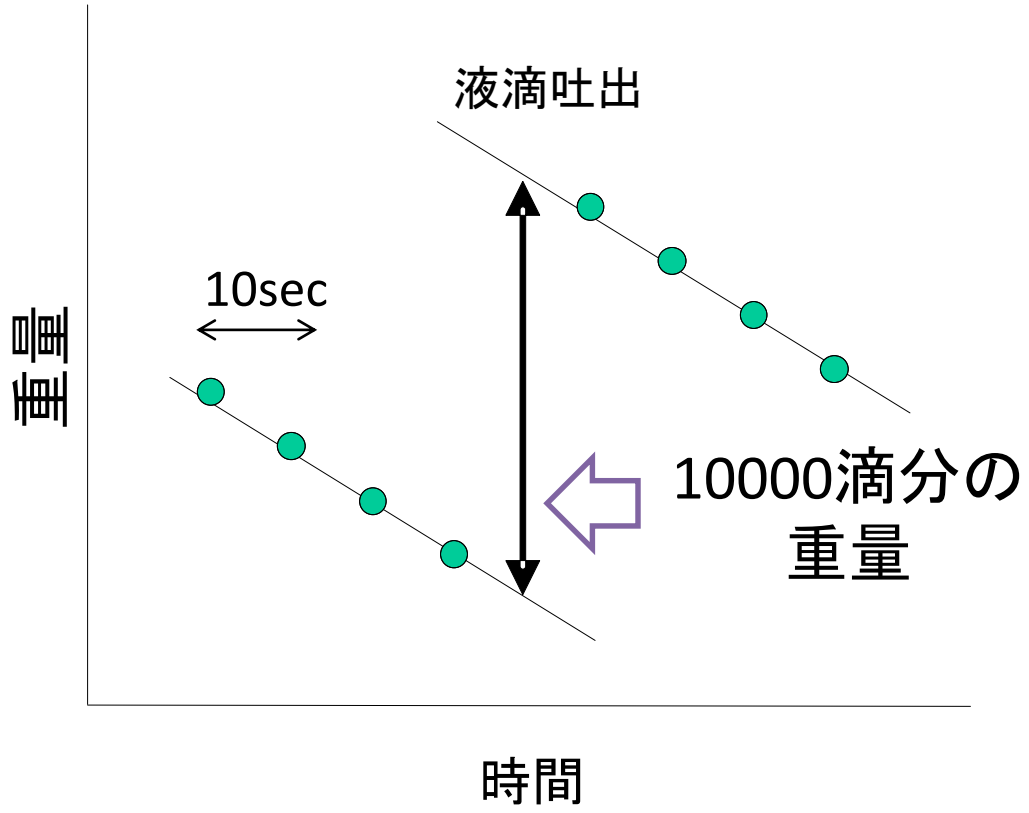


駆動形に依存　→　最適化

液滴重量の算出

①10秒ごとに値を読み取る。
②液滴を吐出する
③再び10秒ごとに値を読み取る。





R.M. Verkouteren et, Anal. Chem. 2009, 81, 8577–8584.

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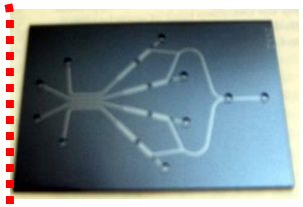
Micro analytical method with droplet

96 holes plate

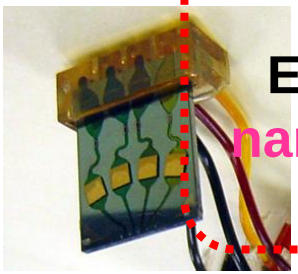
Slow reaction
in open chamber

μ-TAS

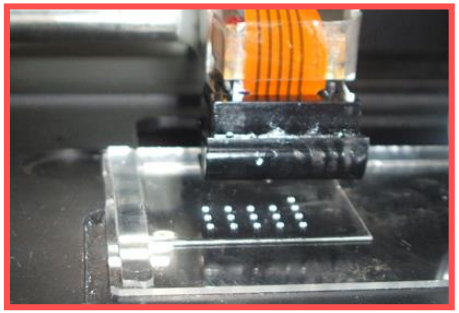
Fast reaction
in closed channel



Ink-jet device

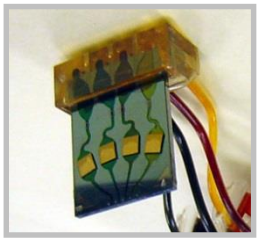


Ejection of
nano-volume
sample

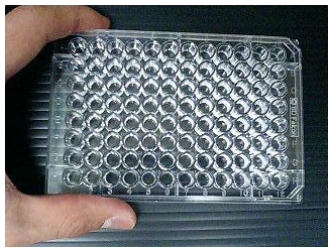


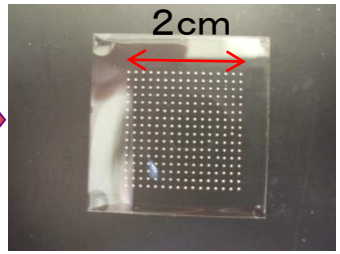
"Open-type" μ-TAS system
Fast reaction in microreaction chamber
(= nano~pico liter droplet)
made by an ink-jet microchip.

Characteristics for droplet as a reaction vial



Pipette(～μL) → Ink jet(～sub nL)





μ-titer plate(sub mL) → Droplet(～sub μL)

	Conventional	Proposed	Effect
Sample amount	～20μL	～20nL	1/1000
Time	～5 h	～5 min	×60
Sensitivity	1	～15	x5＜
Cost	10$	～1c.	1/100
Parallel meas.	96,384,,,	～1000	x10, x 2
Portability	–	Easy	Hand held

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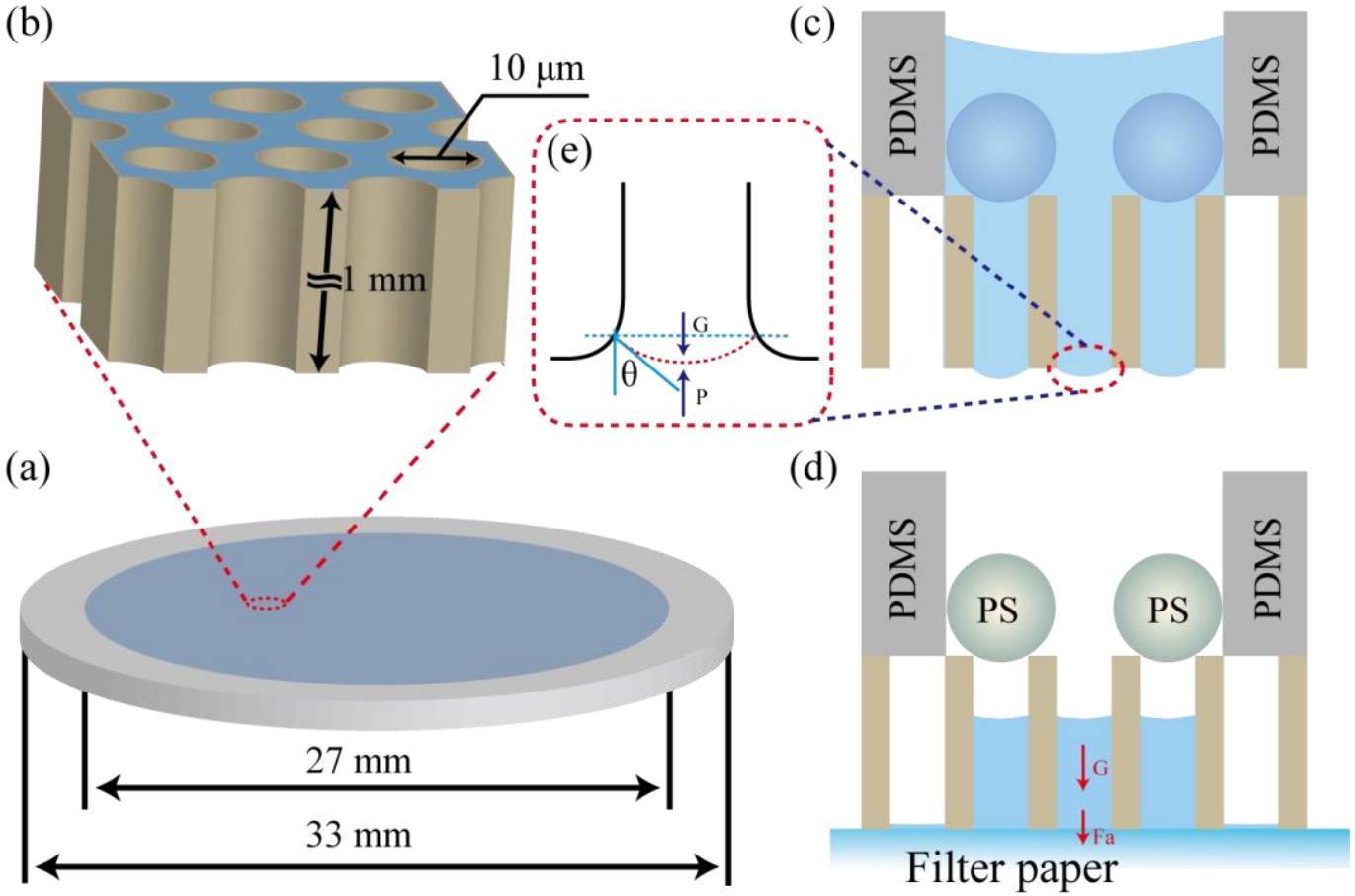
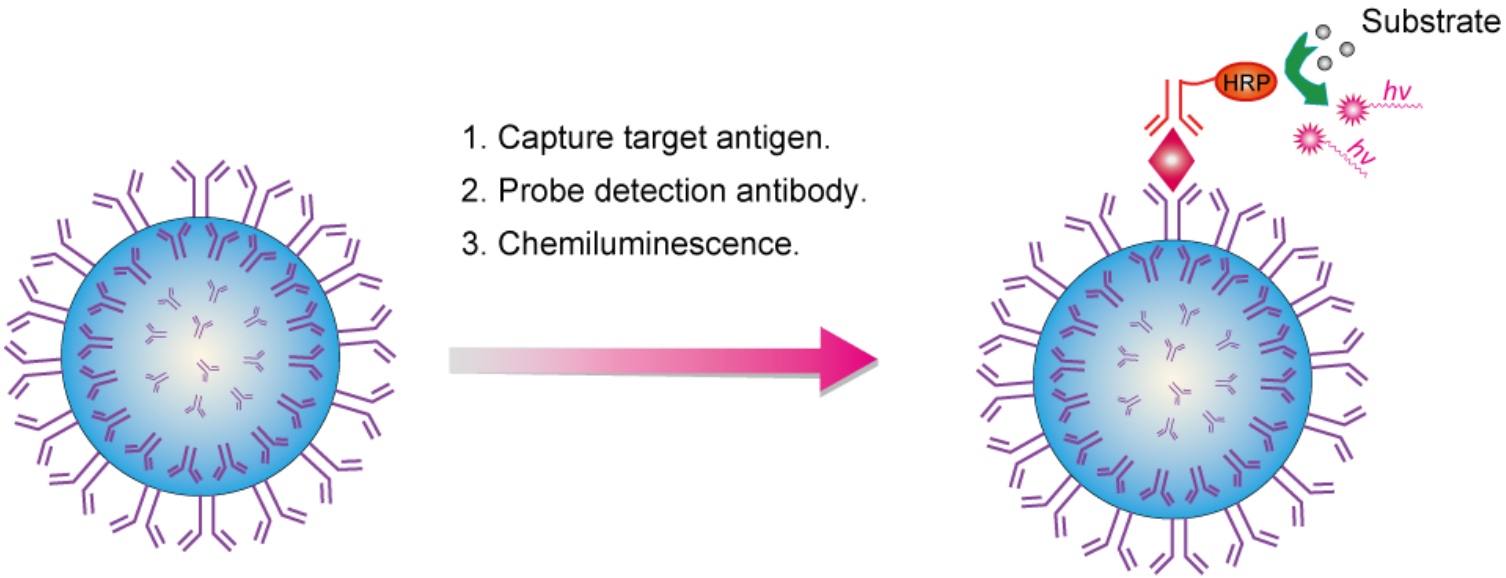
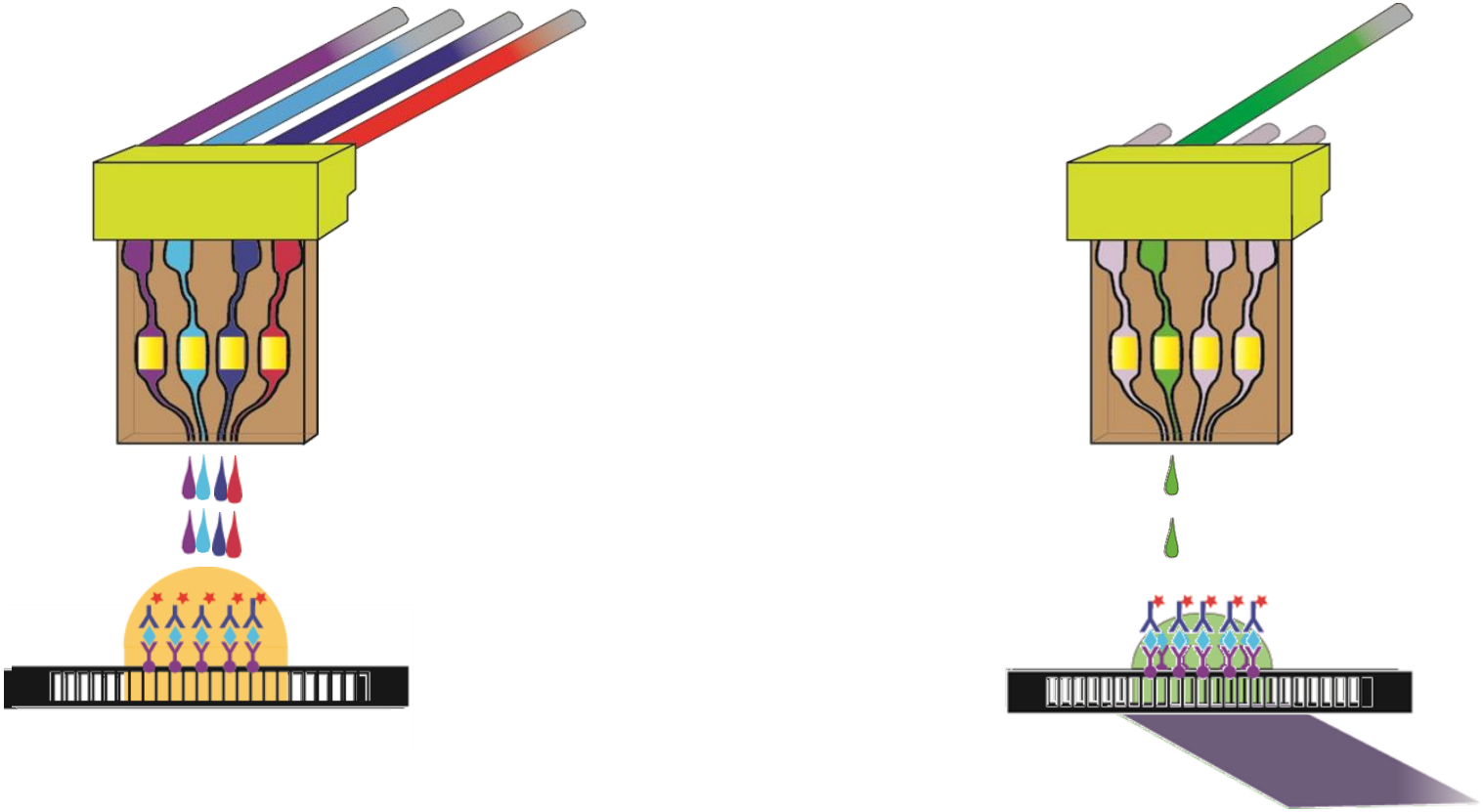
Control holding/passing solution on multicapillary glass

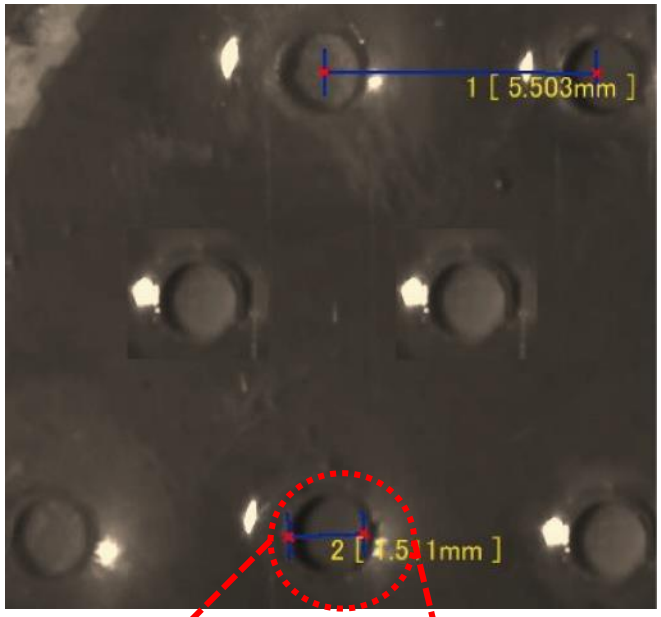


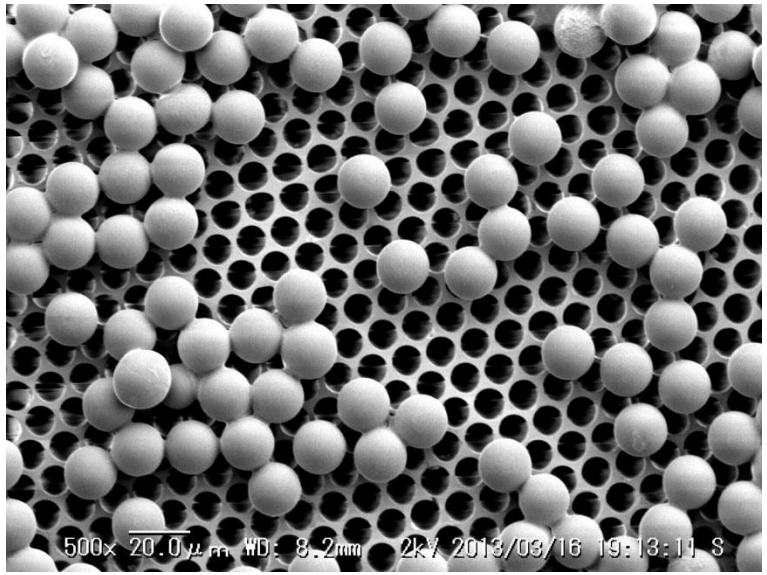
F. Chen, H. Zeng, H. Nakajima, K. Uchiyama, J.-M. Lin, *Anal. Chem.*, 2013, **85**, 7413-7418


1. Capture target antigen.
2. Probe detection antibody.
3. Chemiluminescence.
Substrate
HRP
hv
hv

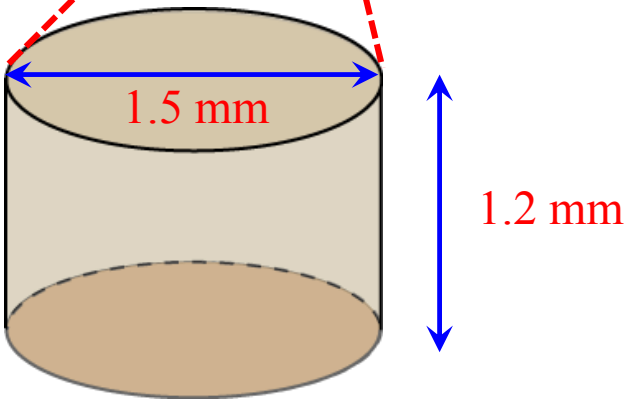


PS-beads on the MCP





SEM image of PS micro beads on the MCP.



Size of each microwell

Experiment setup

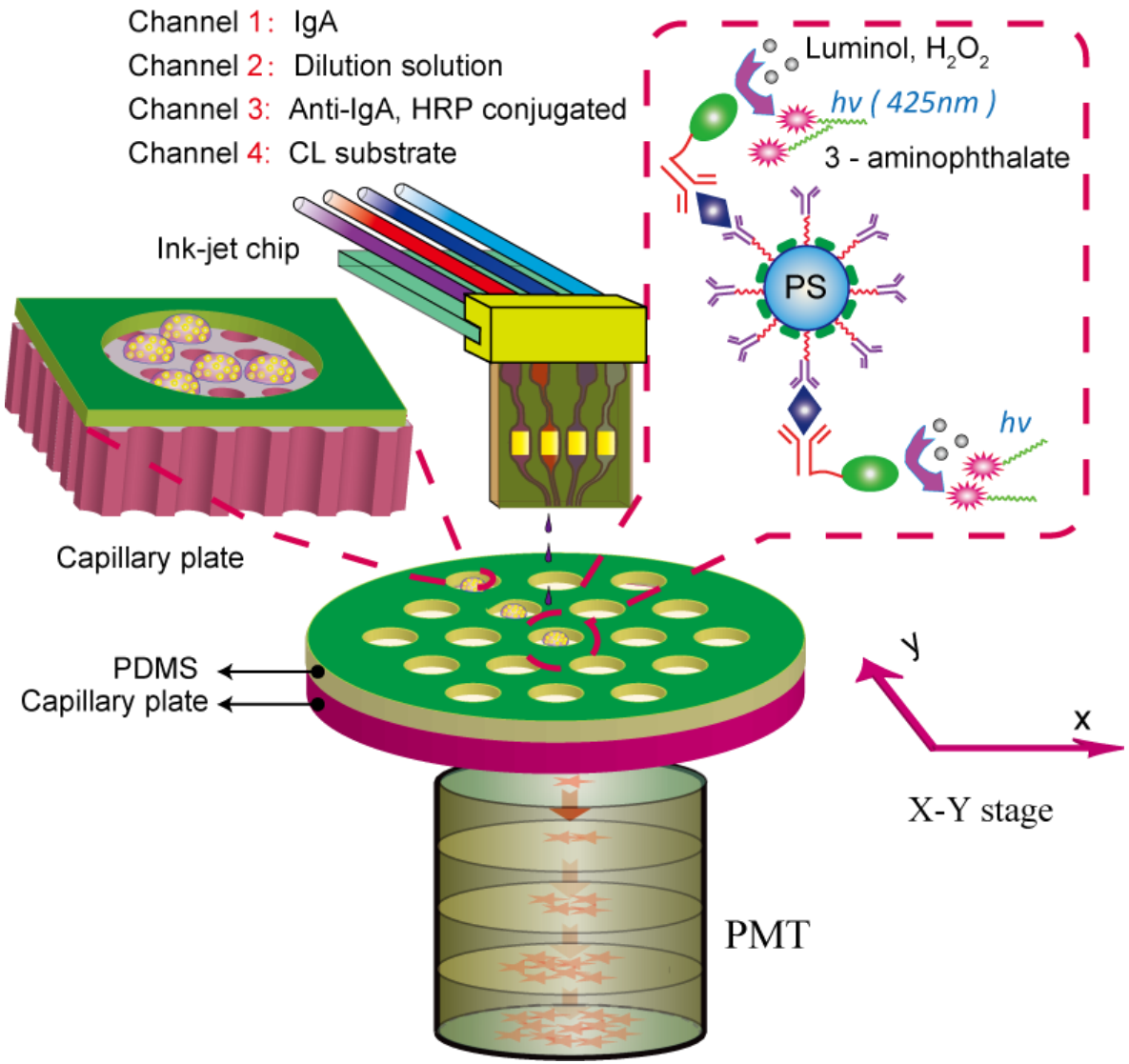
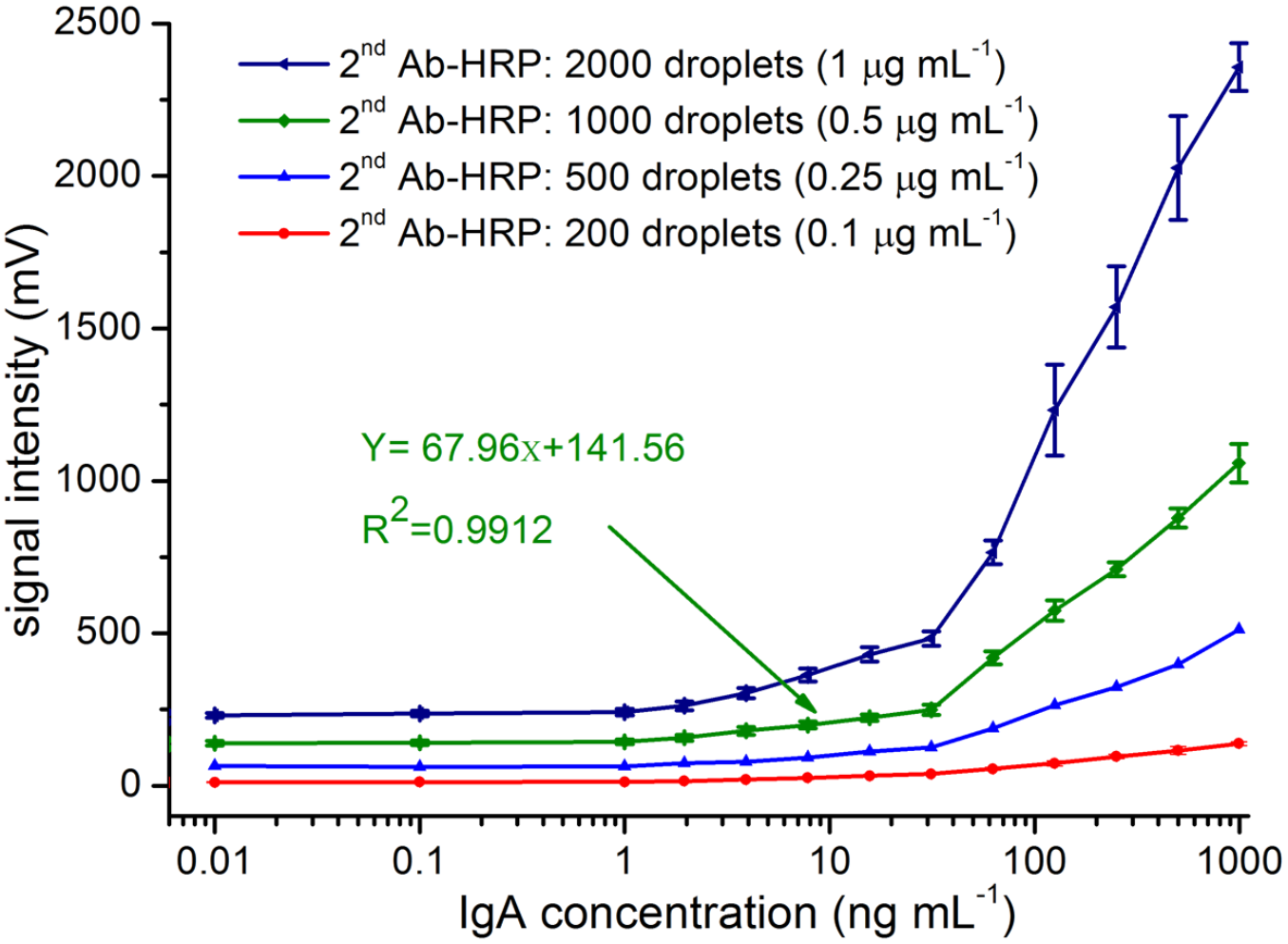


Fig.2 Experiment setup

Results and Discussion



Effect of the concentration of 2ndAb-HRP on the CL signal intensity

Comparison of the assay of human IgA in the multicapillary glass plate with the microwell array and in the 96-well plate.

Methods	Incubation time (min)		Total assay time (min)	Sample volume (μL)	LOD (ng mL^{-1})
	Human IgA	2^{nd} Ab-HRP			
Multicapillary plate	25	25	<60	0.79	0.10
96-well plate	60	60	>140	100	0.86

summary

1. We established a novel chemi-luminescence diagnosis system for high-throughput human IgA detection by inkjet nano-injection on a multi-capillary glass plate change the solution and wash to separate bonded and free antibody (or antigen) (B/F separation).

2. The platform had the advantages of high speed and low reagent consumption. Because of the use of inkjet technology, the platform also had the advantage of potential automation and compaction.

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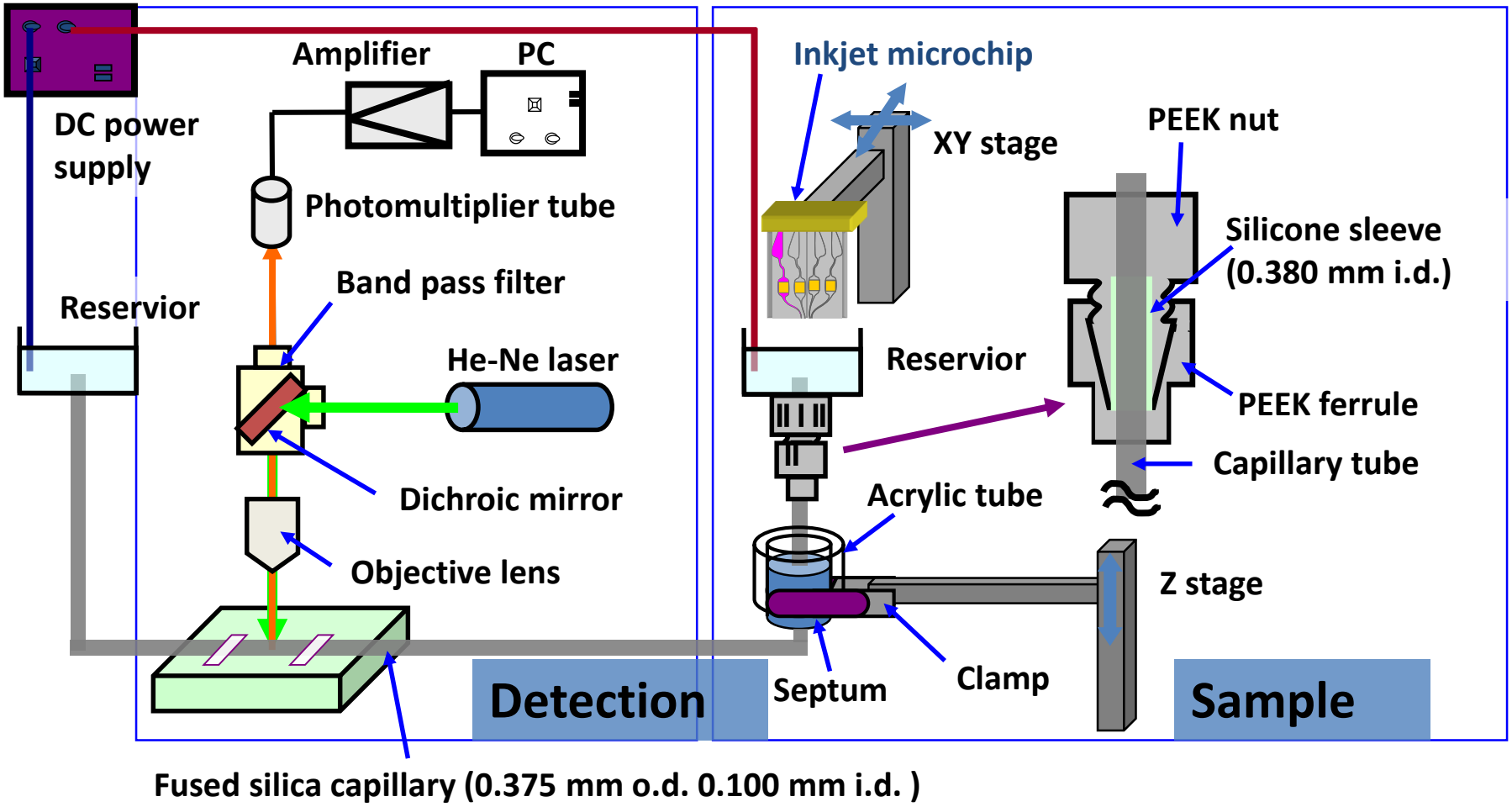
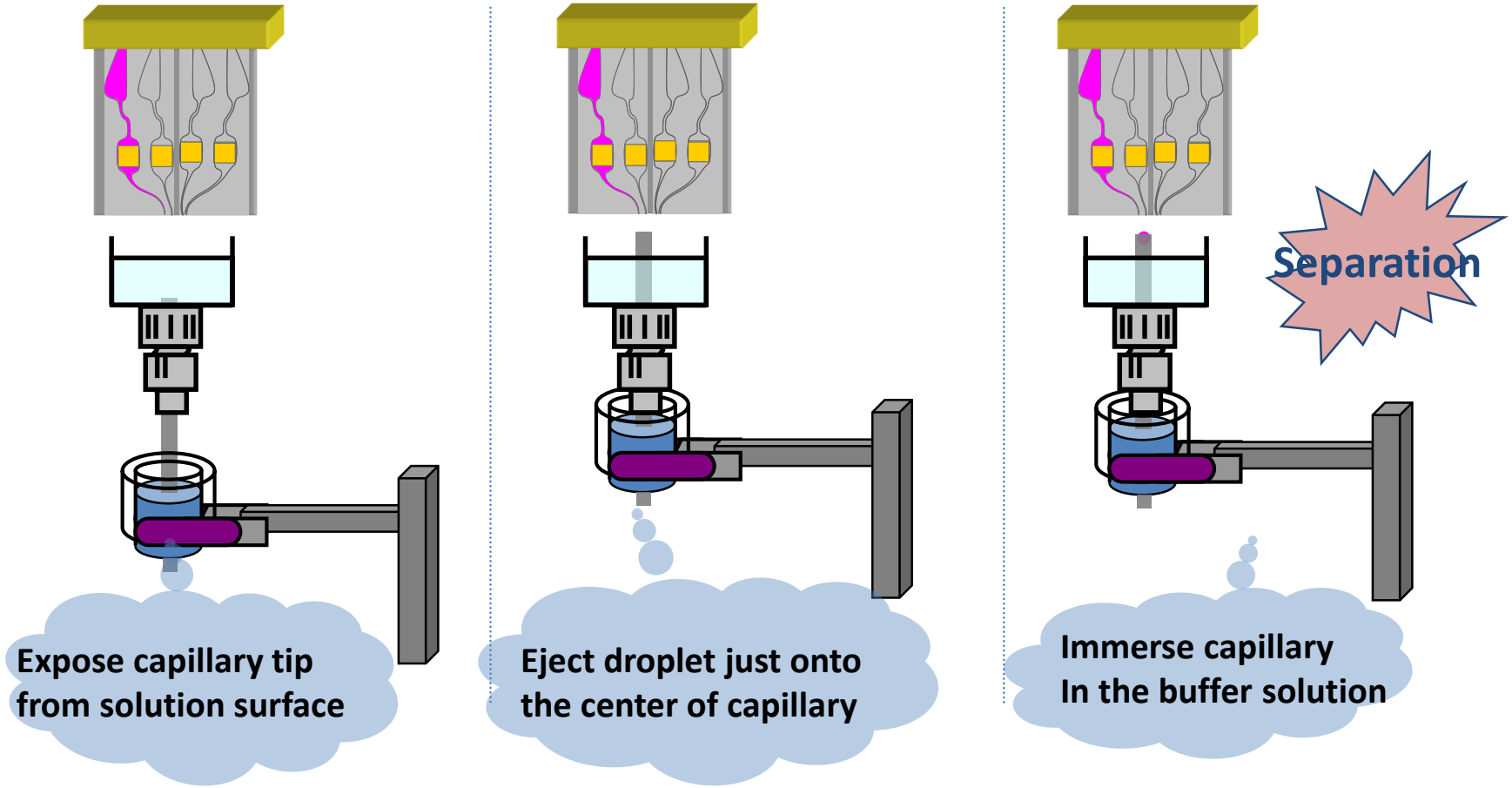
Ink jet introduction

- Sample introduction system



Ink-jet introduction

- How to inject pL sample droplet



Quantitative Electrophoresis Mediated Micro-Analysis (EMMA) by Drop-by-Drop introduction

EMMA

Advantage

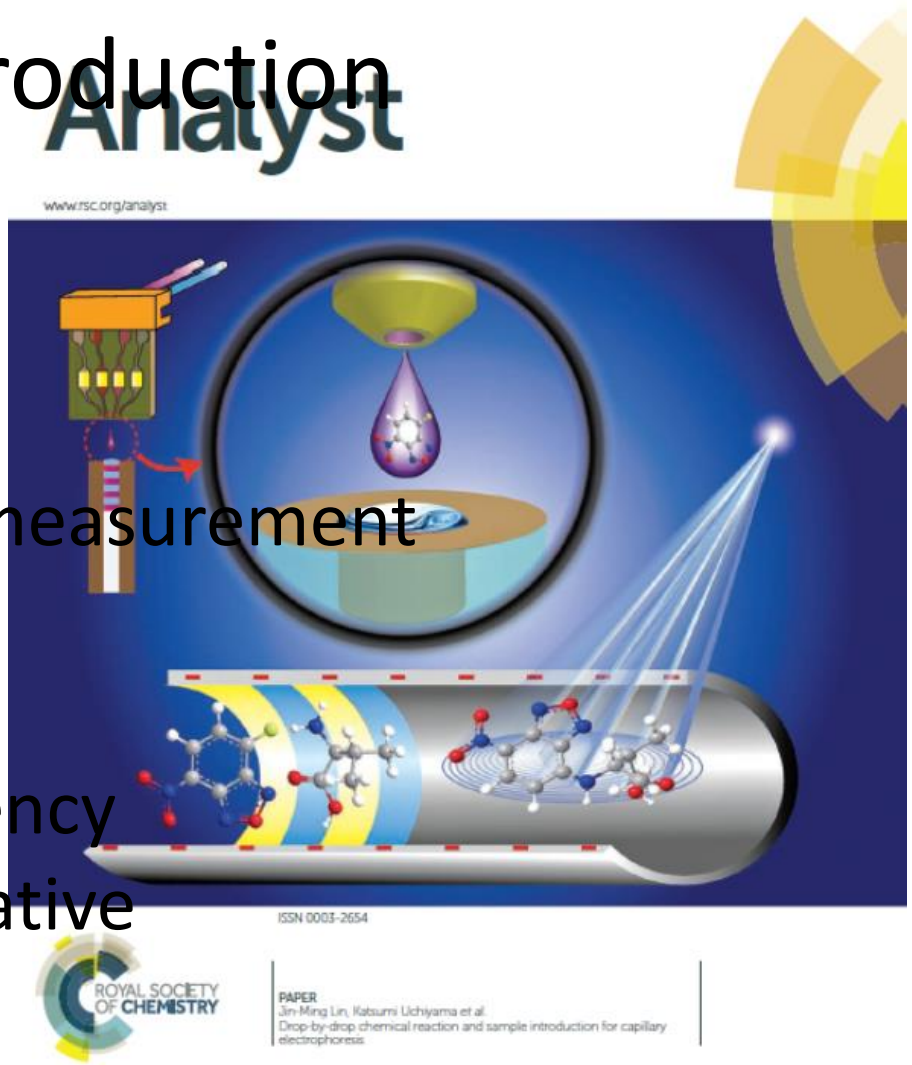
1) Easy to automatic measurement

2) Reproducible

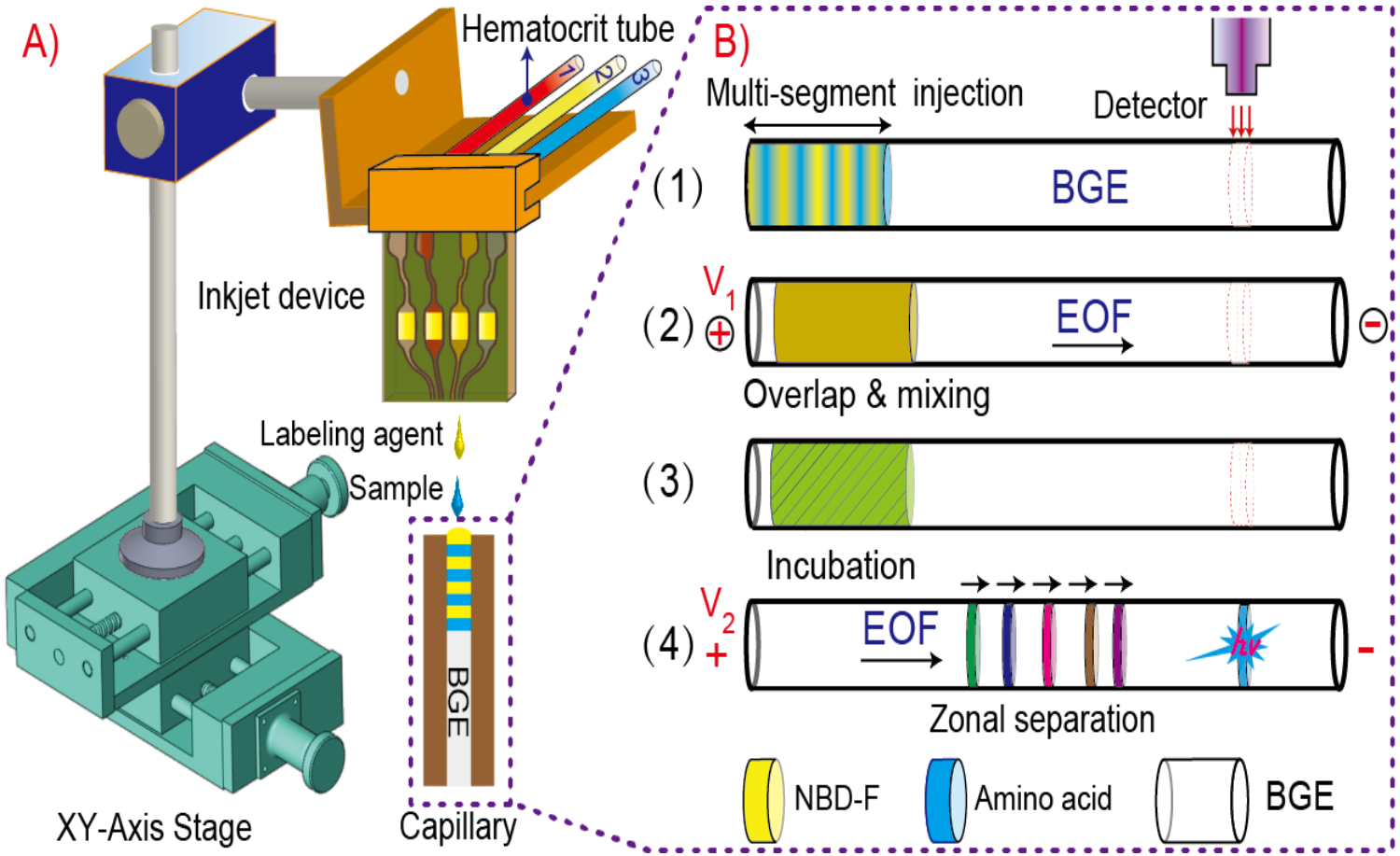
Disadvantage

1) Low reaction efficiency

2) Not always quantitative

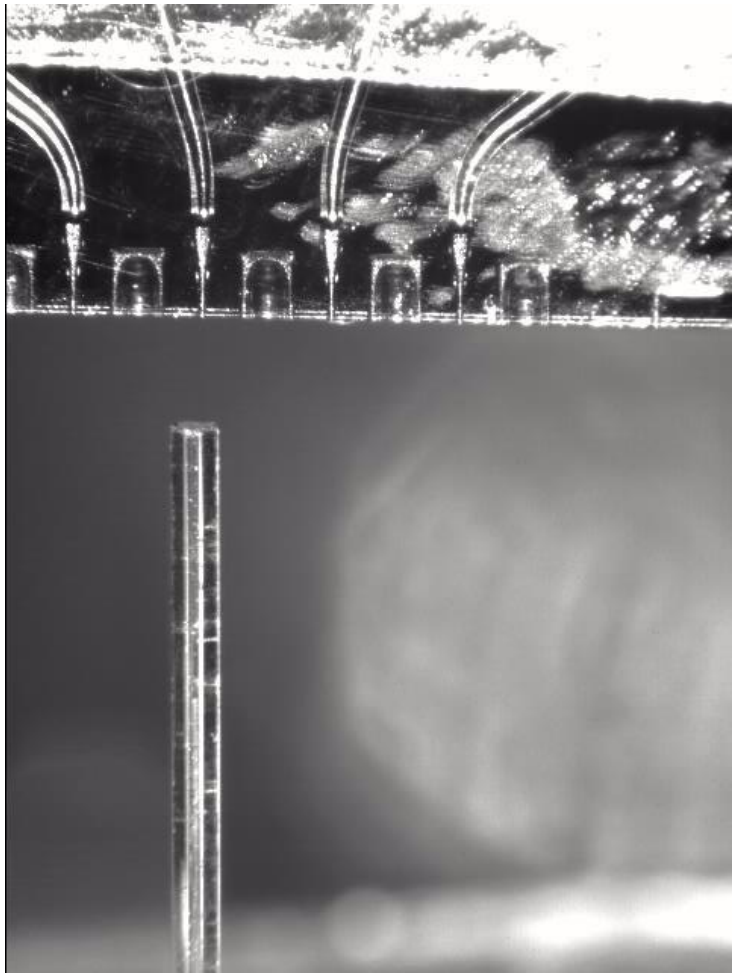


Analyst, 2015, **140**, 3955.

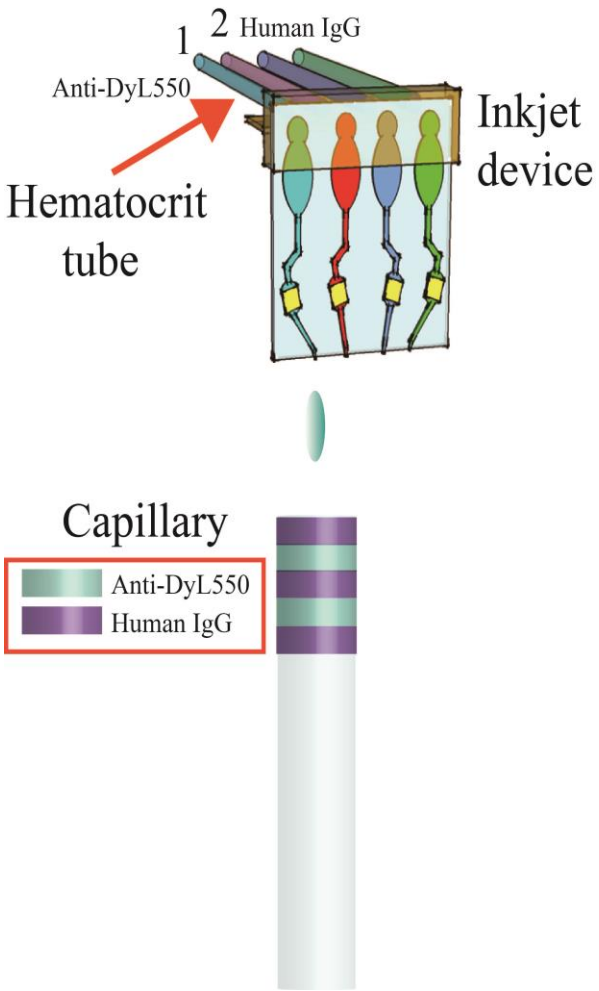


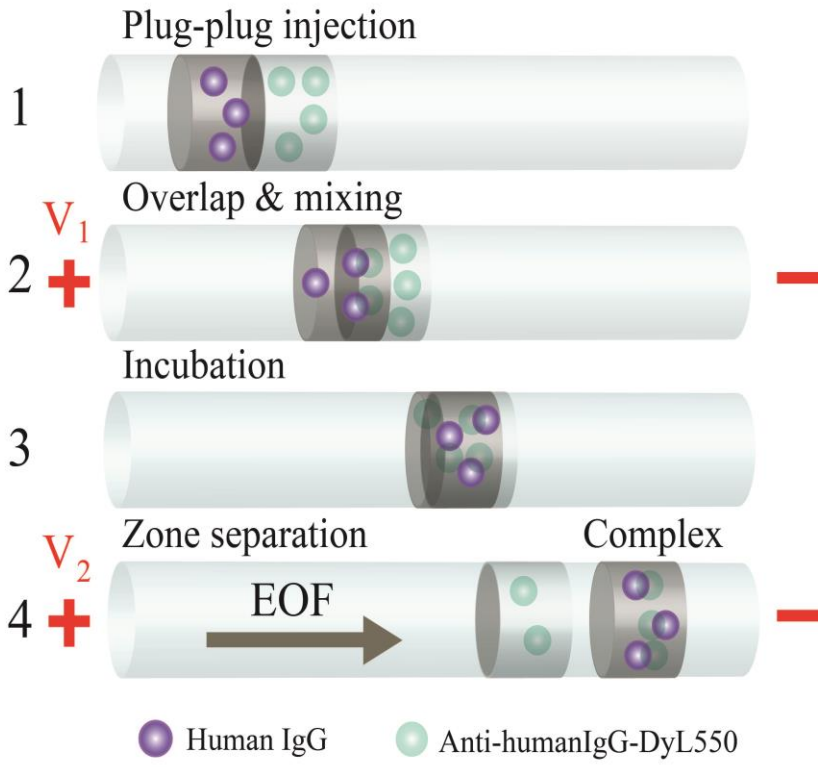
A) Diagram of the inkjet multi-segment introduction system for CE. B) Scheme for the drop-by-drop introduction process for EMMA. (1)Multi-segment injection pattern of sample and reagent, (2) Overlapping of zones, (3) Incubation for labelling reaction, (4) Separation by electrophoresis and detection.

Alternate sample/reagent introduction by inkjet

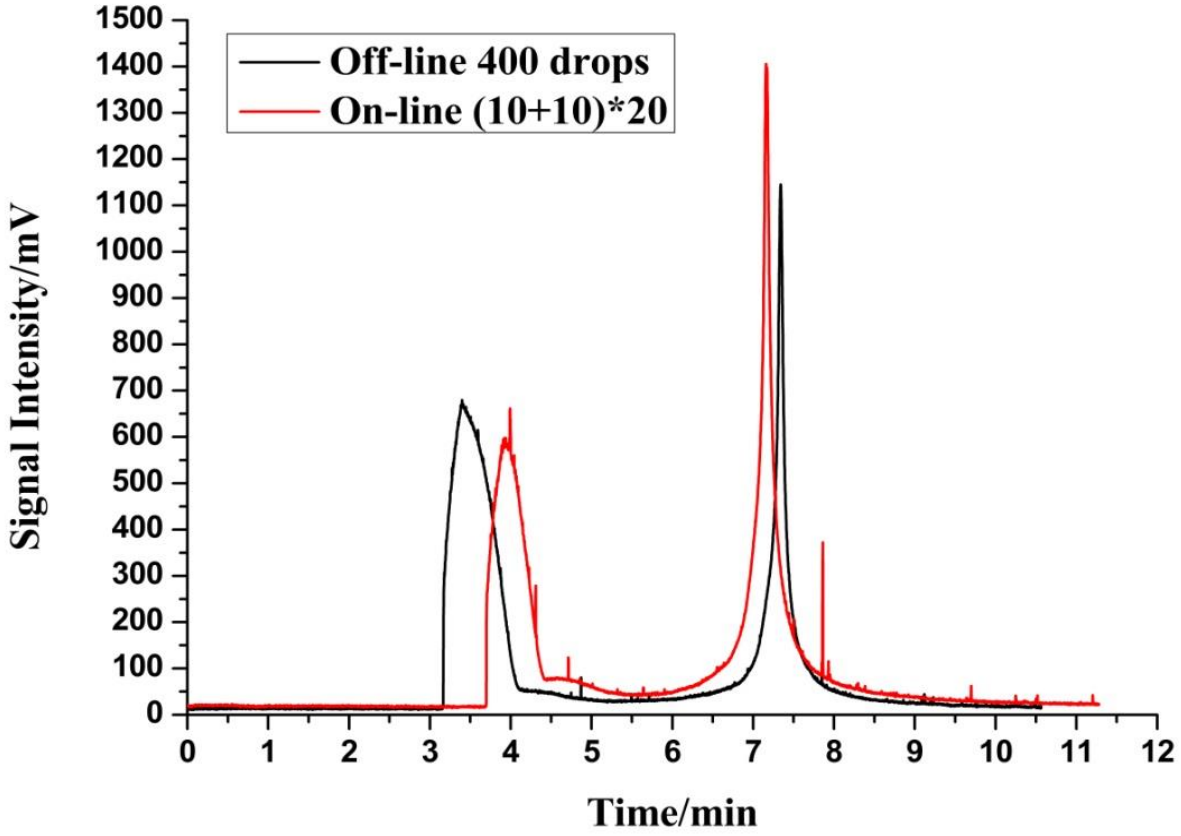


EMMA for immune reaction

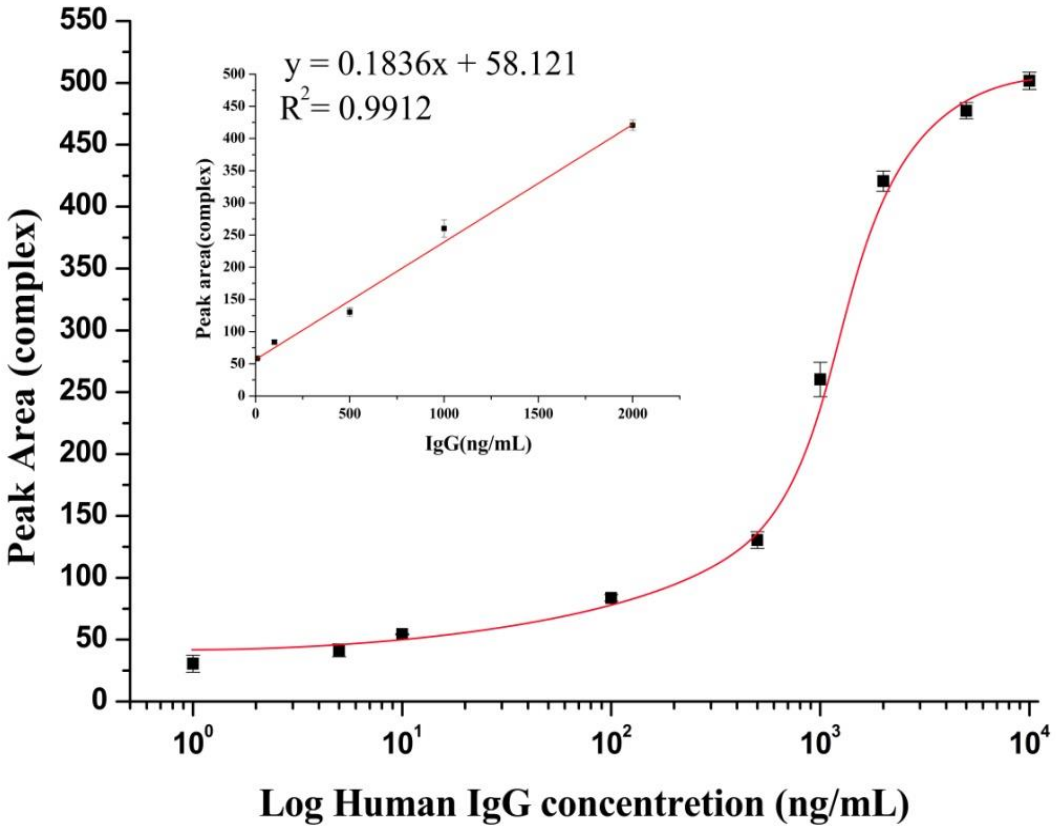




Comparison of the Electropherograms of the immune complex



Calibration curve for IgG



Detection Limit
5ng/mL IgG

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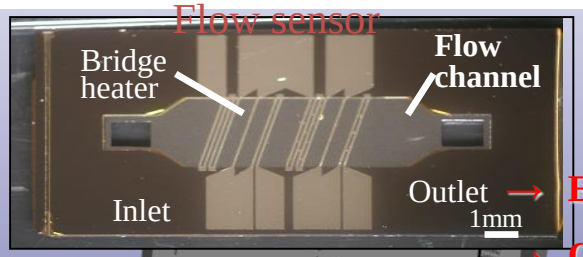
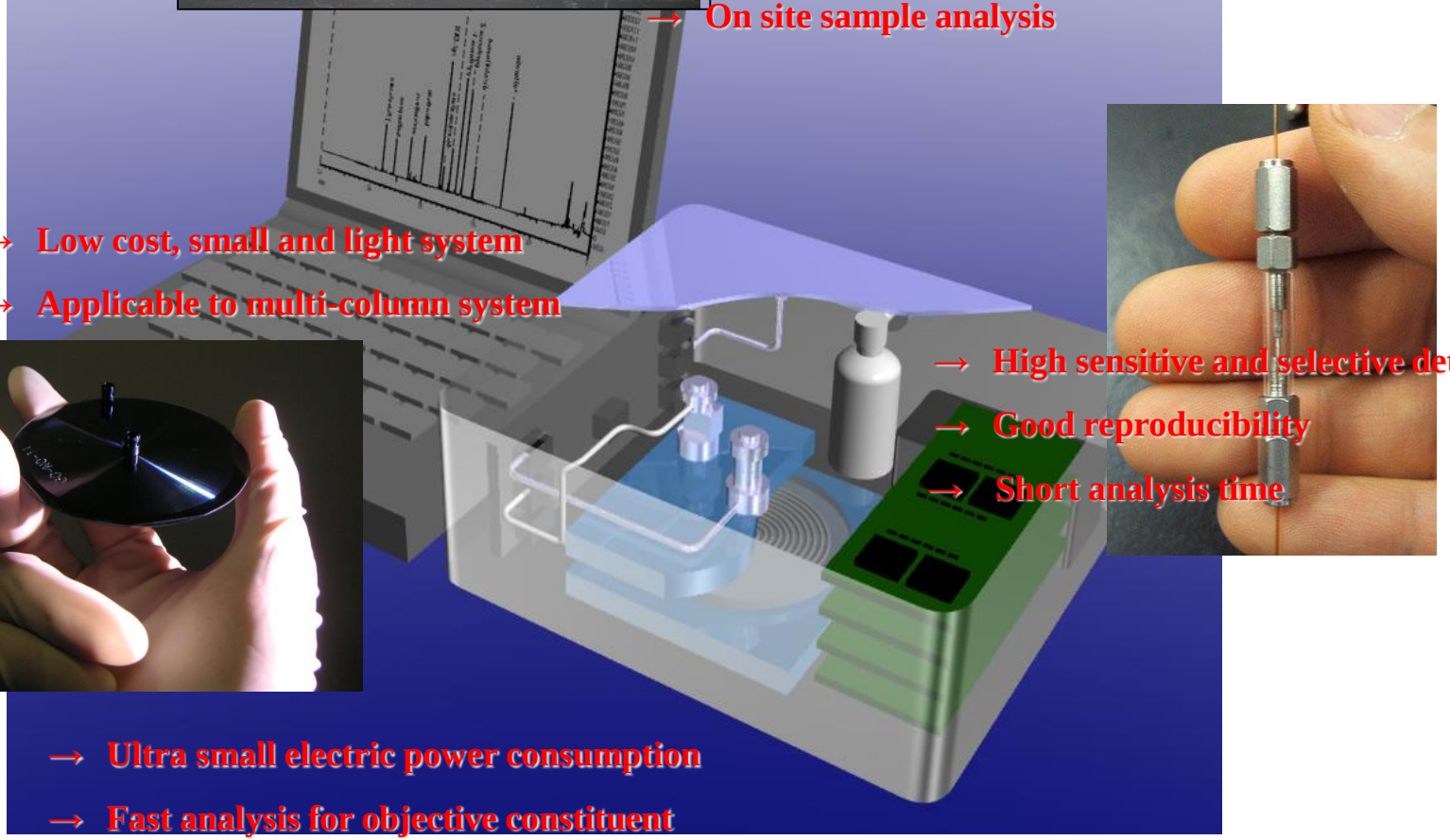
Micro GC system for *on site* analysis



→ Easy connection to the pre-treatment devices

→ On site sample analysis



→ Low cost, small and light system

→ Applicable to multi-column system

→ High sensitive and selective detection

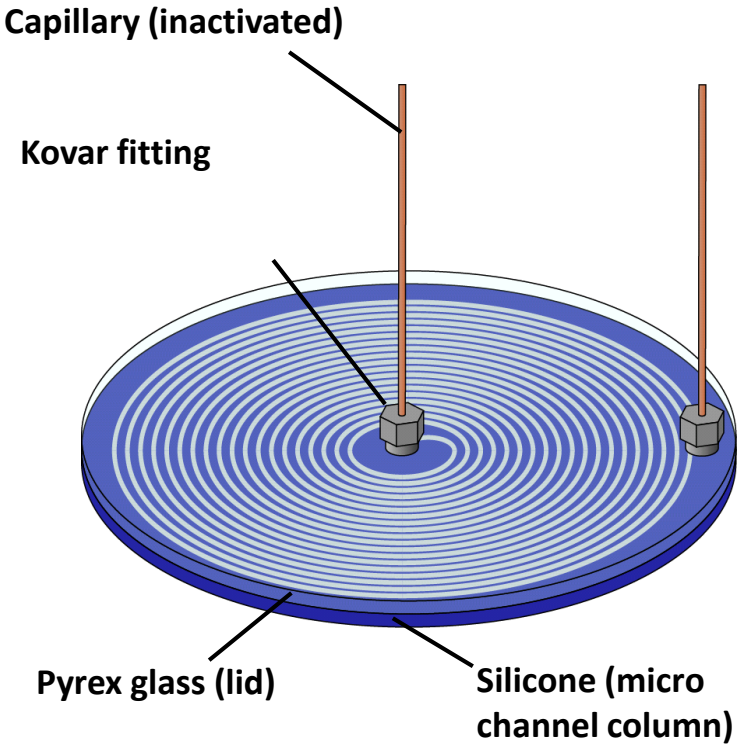
→ Good reproducibility

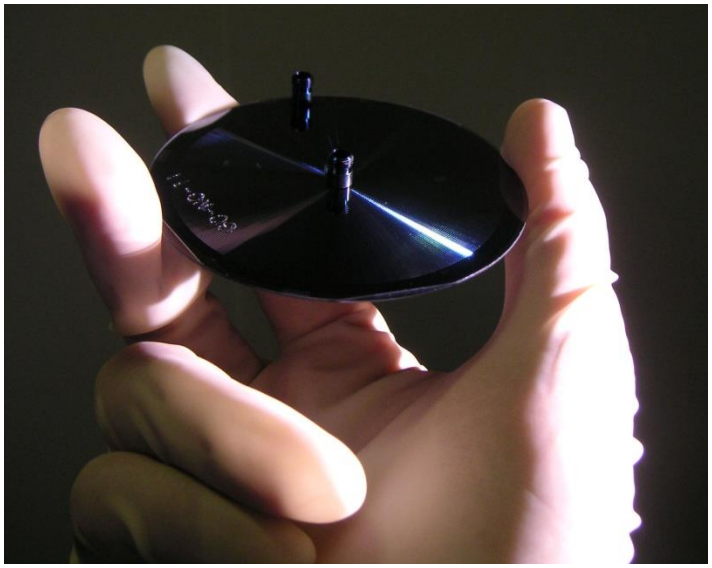
→ Short analysis time

→ Ultra small electric power consumption

→ Fast analysis for objective constituent

Structure of On chip column

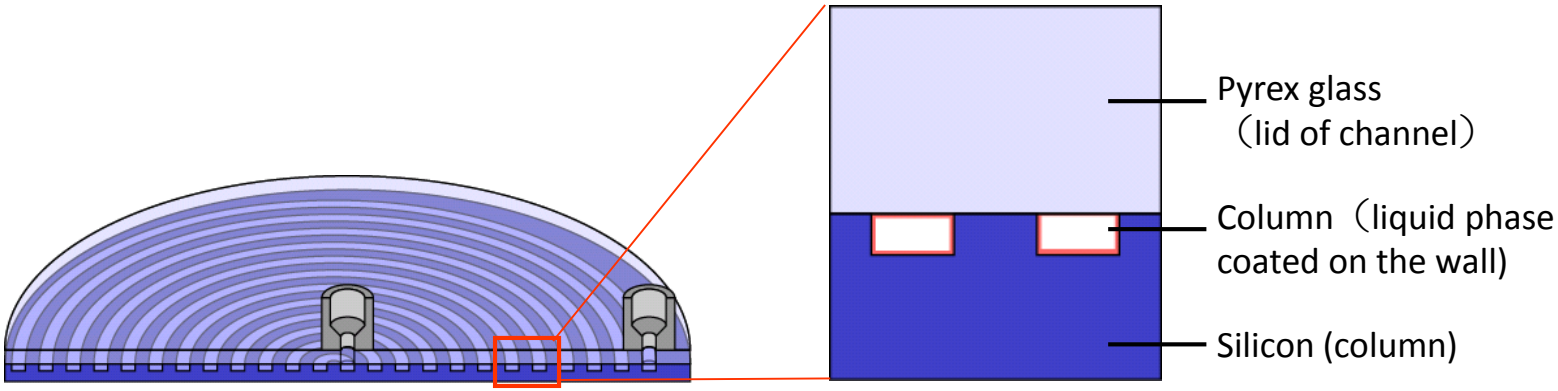




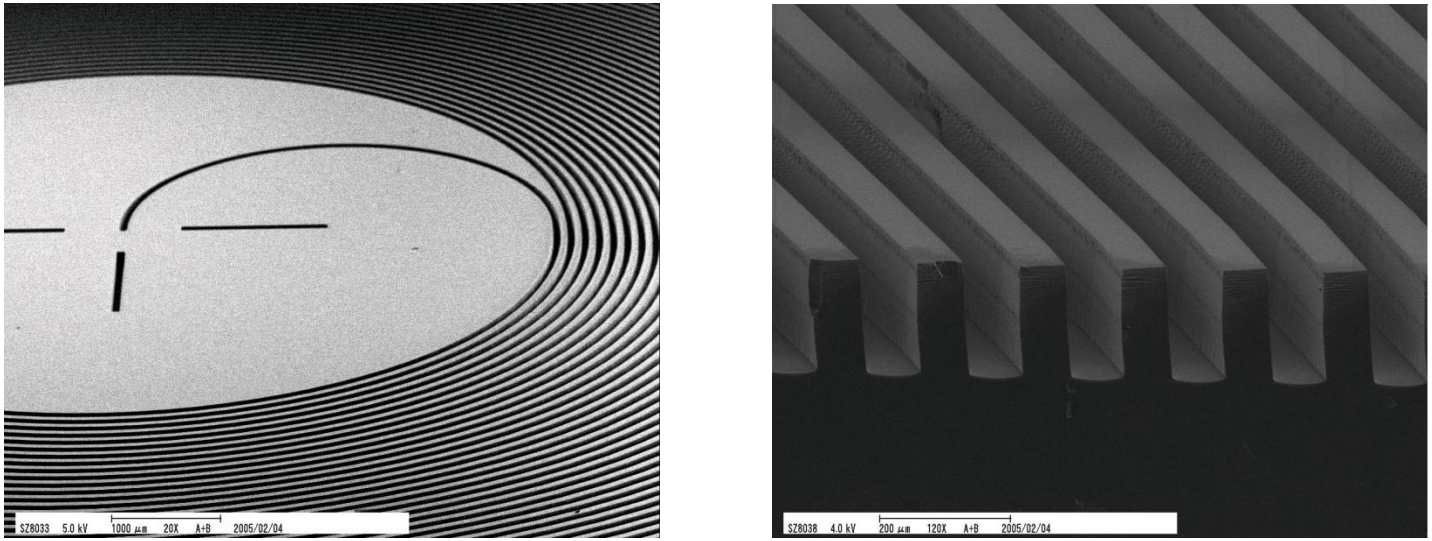
External view of on chip column (φ76mm)

- Silicon substrate was dry-etched to form spiral micro channel with 50～200μm width and ~100μm depth.
- Three kinds of liquid phase (**100% poly-siloxane**, **5% Phnyl / 95% Poly-siloxane**, **Polyethylene glycol**) was coated by vacuum process and evaluated by normal c-GC system.

On chip column unit



Cross section of the column



SEM image of the channel, Width; 50μm, Depth; 100μm

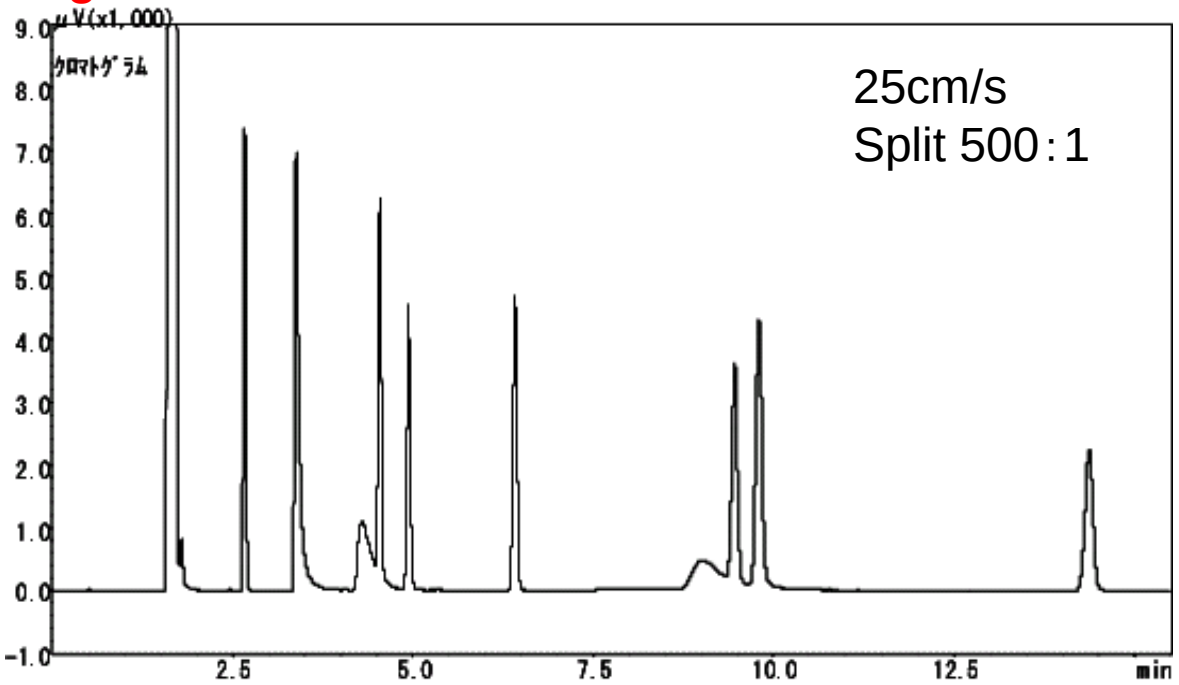
(Left: external view, Right: magnified view of cross section

Development of chip column with high resolution

- column: 100 μm in width and depth, 17m in length

  Large theoretical plate number >78,000

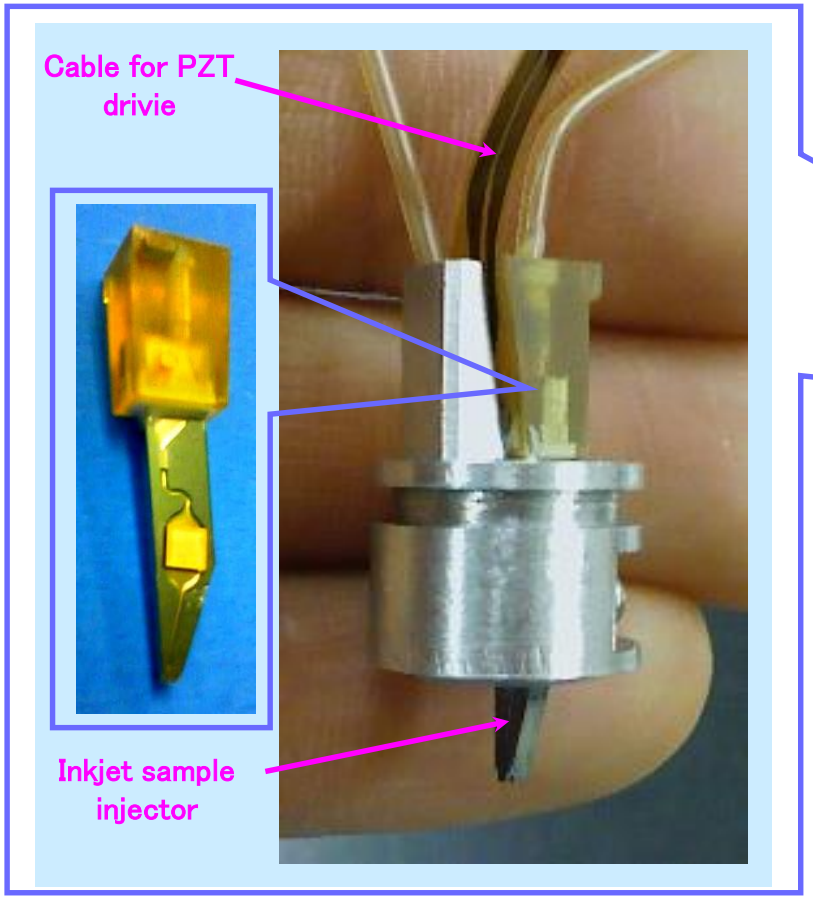
World highest record.

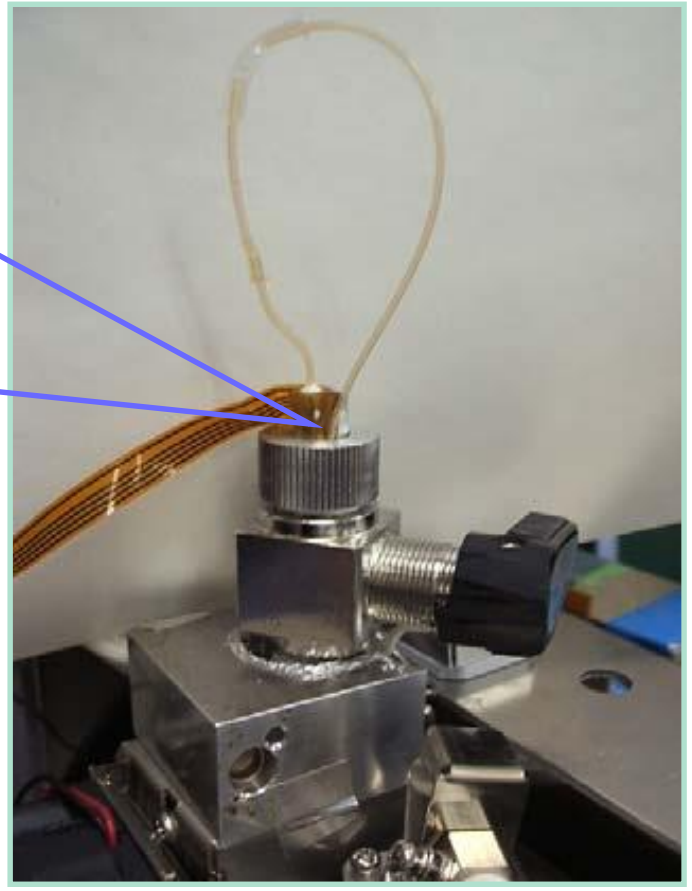


Relative retention	Df(nm)	Teroretical plate number	HETP/m
7.826	261 (estimated)	78,785	4634

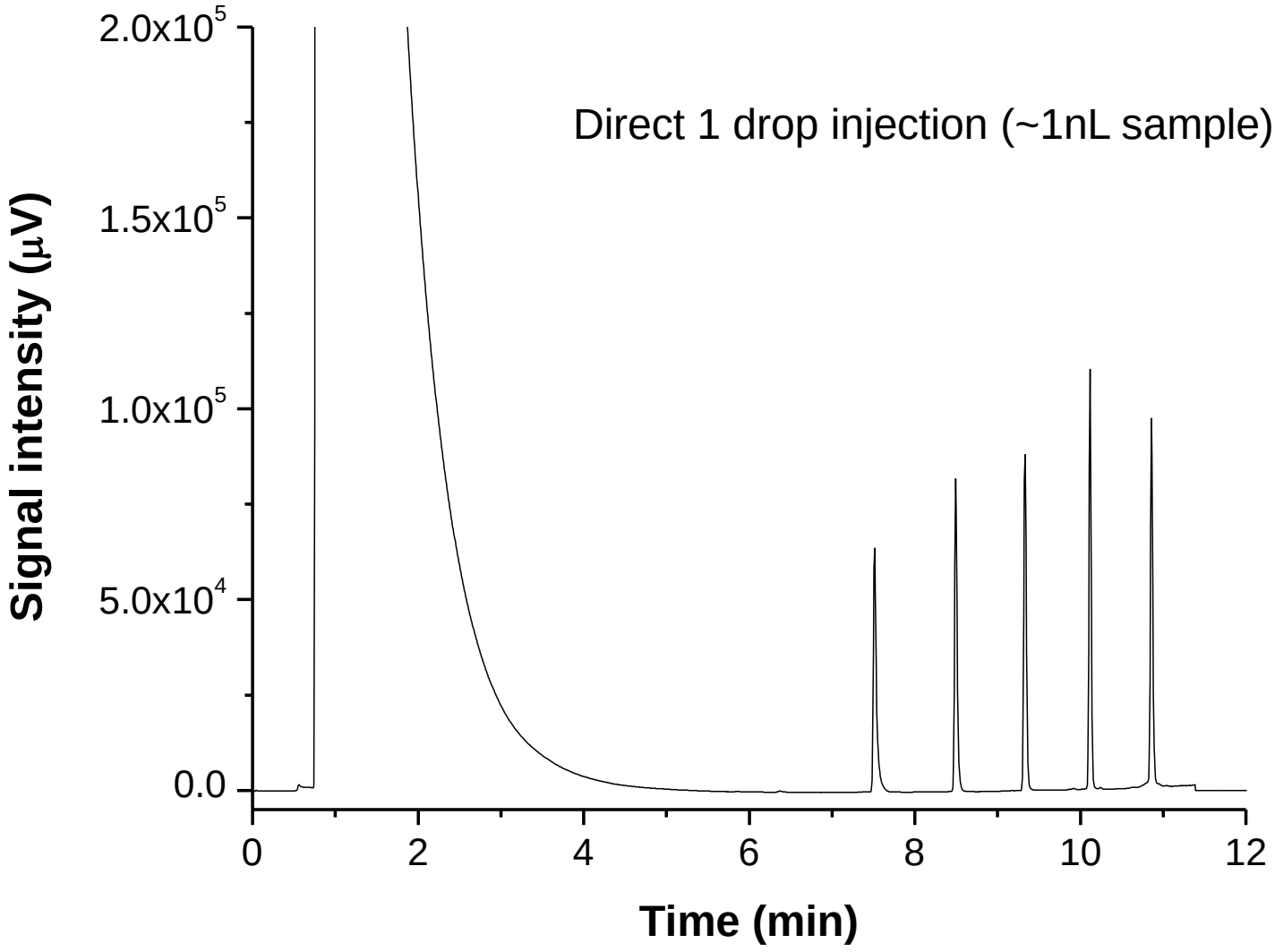
Inkjet sample injector with pressure feed back

- Ink jet micro chip was applied to GC injection port
- Pressure at the nozzle tip was fed back to sample loop
- Reproducible sample introduction of nano-liter amount was successfully carried out in the pressure range of 0 to 10kg/cm^2 at injection port.

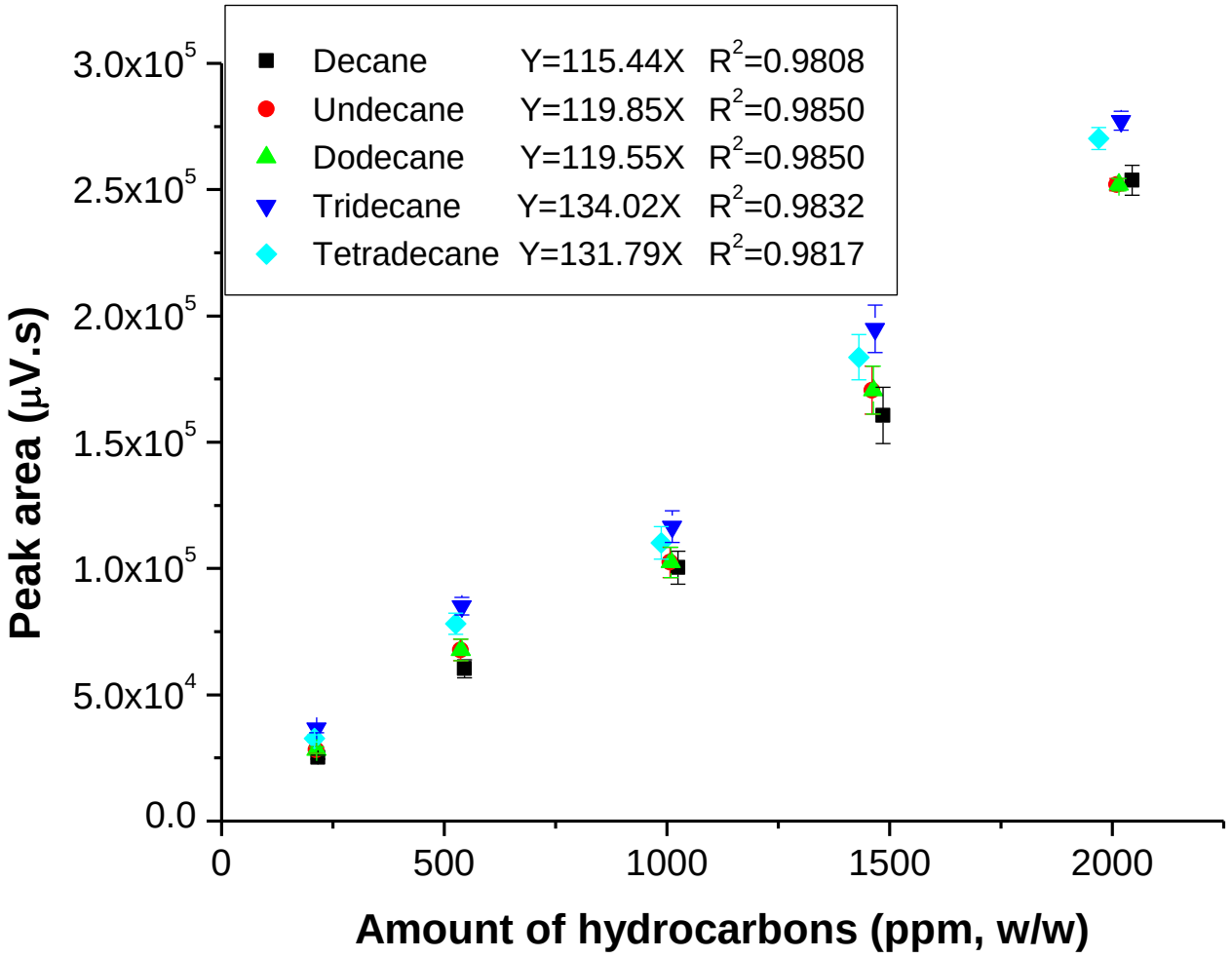




Chromatogram for hydrocarbons



Calibration curve for hydrocarbons introduced with ink-jet



本発表の内容

1 インクジェットの原理

2 微小液滴を利用したバイオアナリシス

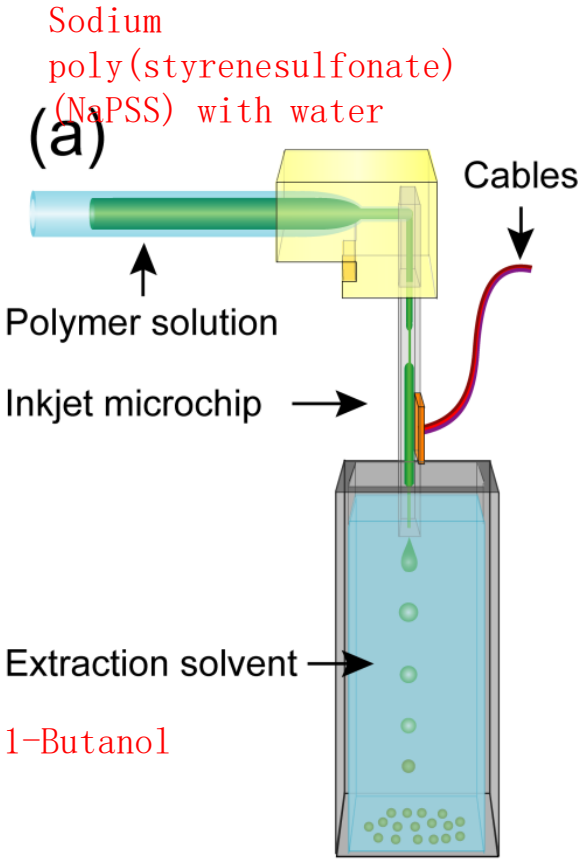
3 インクジェットを用いたELISA

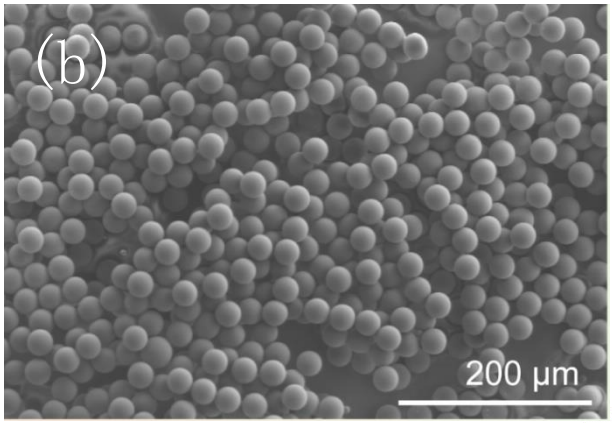
4 キャピラリー電気泳動分析への応用

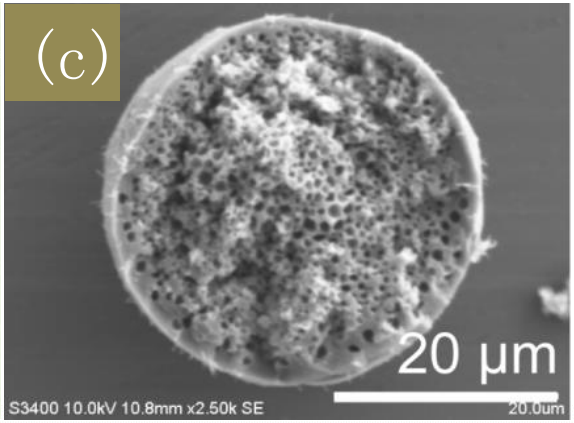
5 ガスクロマトグラフィーへの応用

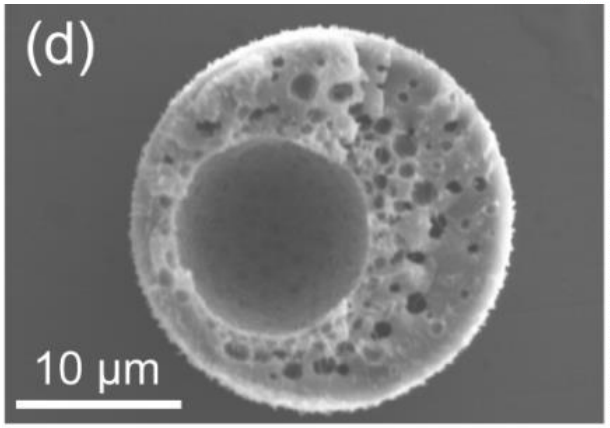
6 インクジェットによる単分散高分子微粒子の生成

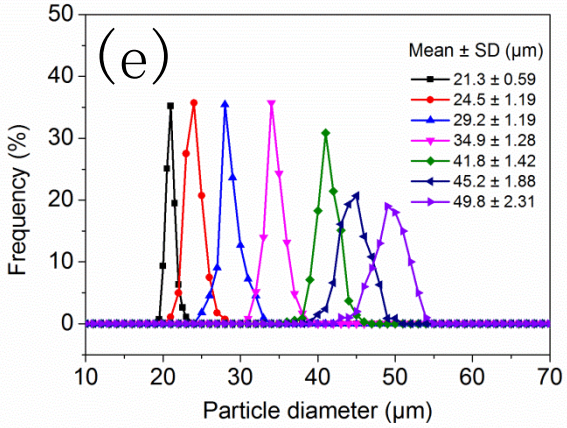
Ink-jetting approach for porous particle formation







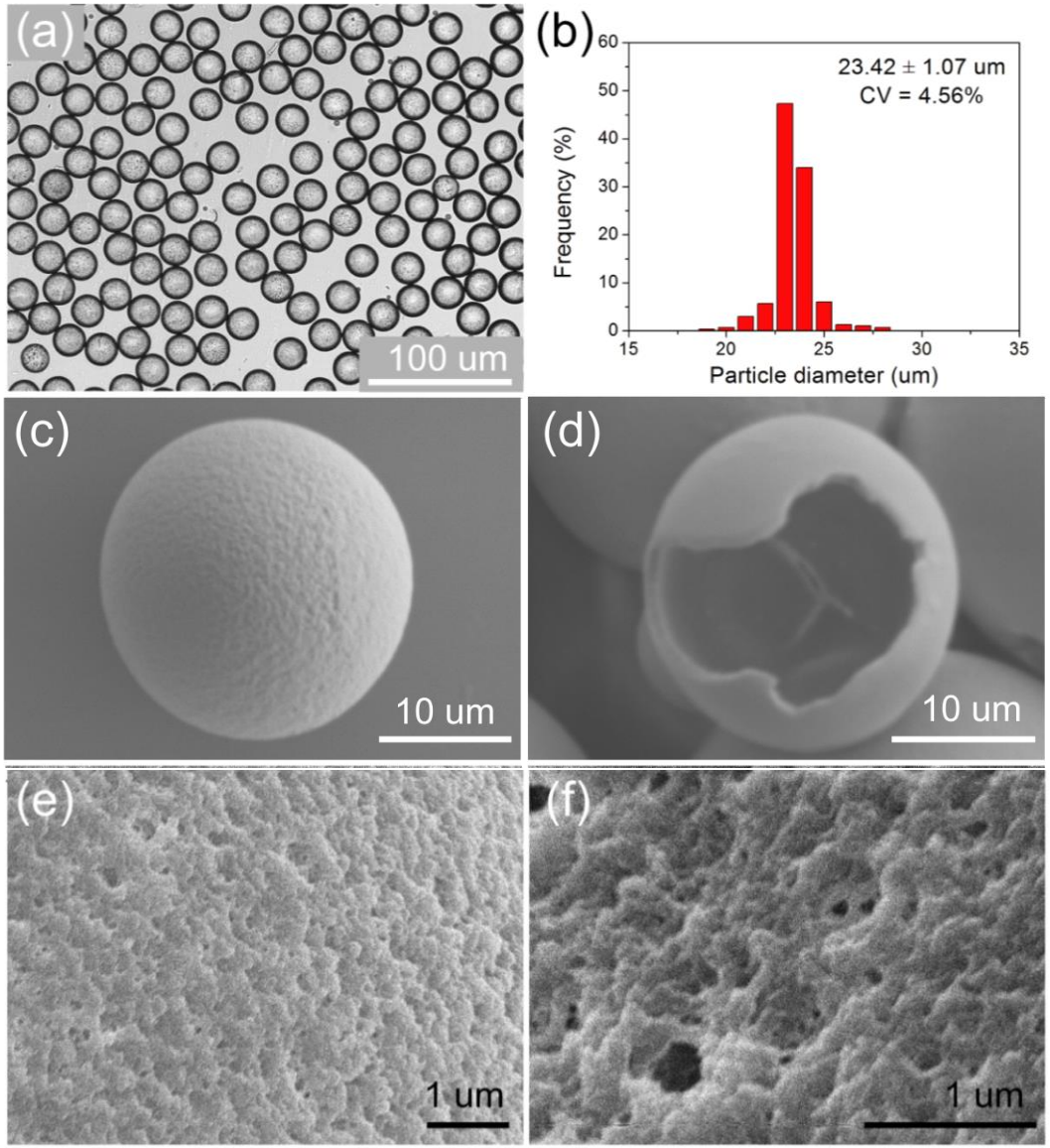




RSC Advances, 2015, **5**, 7297–7303

✓ **Narrow size distributions** ✓**Easily controlling the particle size**

Preparation of hollow core-porous shell HDDA particles



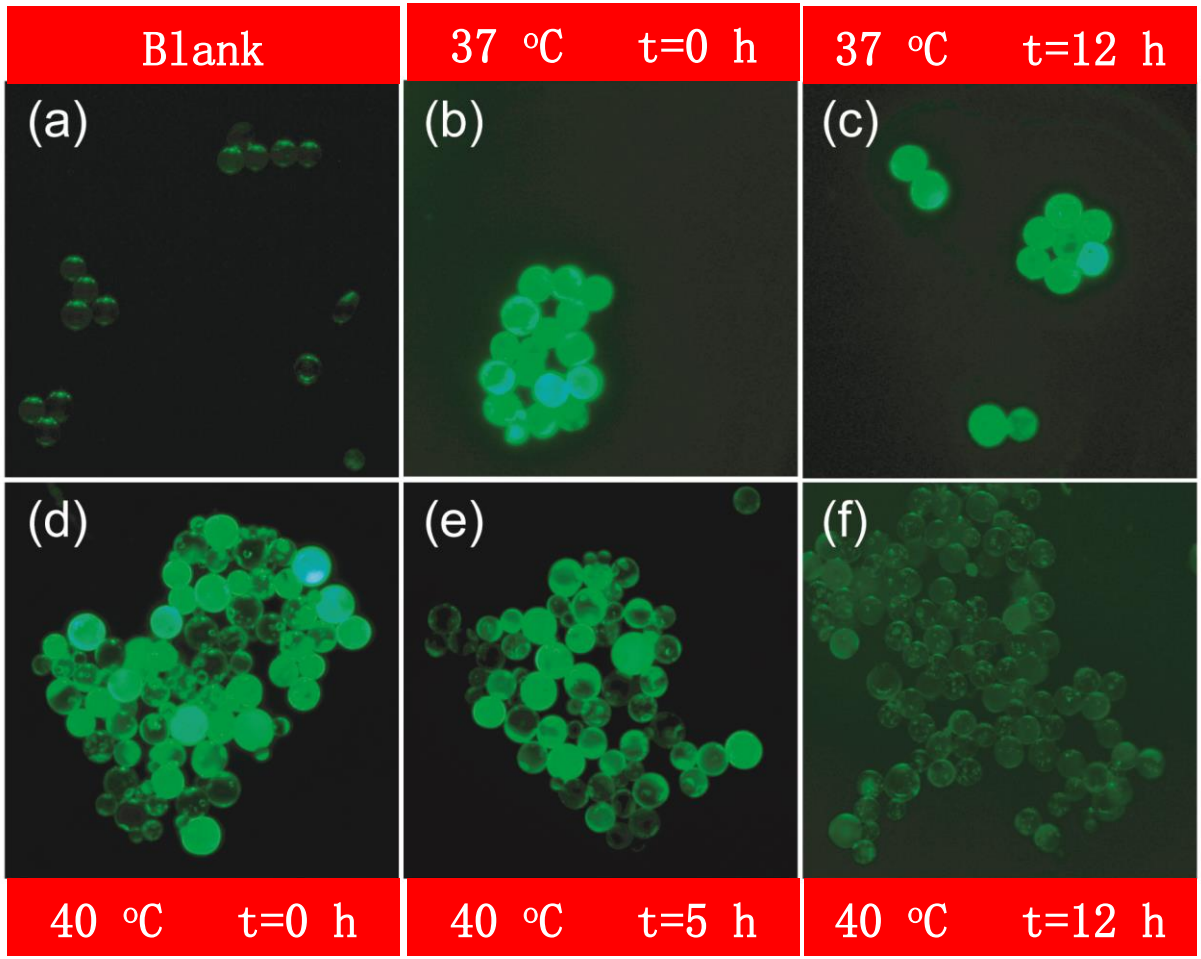
Optical micrograph of the HDDA particles (a) and its size distribution (b).

SEM images of surface (c) and interior structure (d) of HDDA particle.

(e, f) Typical FE-SEM image of the surface of HDDA particles.

Pore size 50-200 nm

Drug release investigation



(a) microspheres without fluorescein
(b, c) microspheres encapsulation fluorescein
(d, e, f) fluorescein release from the microspheres

Students in collaboration and OBs

Our recent Papers related to InkJet

1. *Analytical Chemistry*, 2016, **89**, pp.1342-1435.
2. *Journal of Materials Chemistry* B, 2016, **4**, pp.4156-4163.
3. *Analytical Chemistry*, 2016, **88**, pp 4354–4360.
4. *Journal of Separation Sciences*, 2015, **38**, 2722-2728..
5. Sensors and Actuators B, 2015, **220,** 958–961
6. *The Royal Society of Chemistry Advances*, 2015, **5,** 7297–7303
7. *Analyst,* 2015, **140**, 3953–3959 (Cover)
8. *Sensors*, 2014, **14**, 9132-9144
9. *Chemical Communications*, 2014, **50**, 10265-10268
10. *Analytical Methods*, 2014, **6**,2832-2836. (Rear cover)
11. *Talanta*, 2013, **116,** pp1005–1009.
12. *Analytical Chemistry*, 2013, **85** (15), pp 7413–7418
13. *Chromatography*, 2013, **34**(1), pp.33-40.
14. *Journal of Mass Spectrometry. 2013,* **48**(3), 321-328.
15. *Talanta*, 2013, **107**, 111-117.
16. *Sensors and Actuators* B 2012, **168**, 446-452.
17. *Analytical Chemistry,* 2012, **84**, 10537-10542.

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4) NEDO
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