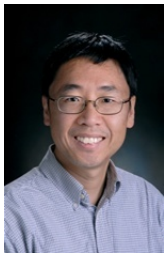
# Acknowledgement



- **Mentors**
  - Profs. Paul Li,
  - Prof. George Whitesides
  - Prof. Richard Mathies
- **Group members**
  - Dr. Peng Zuo
  - Maowei Dou
- **Financial support**
  - NIH
  - UT STARS Award
  - UTEP Start-up, IDR2, URI & CoS MRAP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# Bioanalysis on Microfluidic Lab-on-a-chips

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University of Texas at El Paso (UTEP)

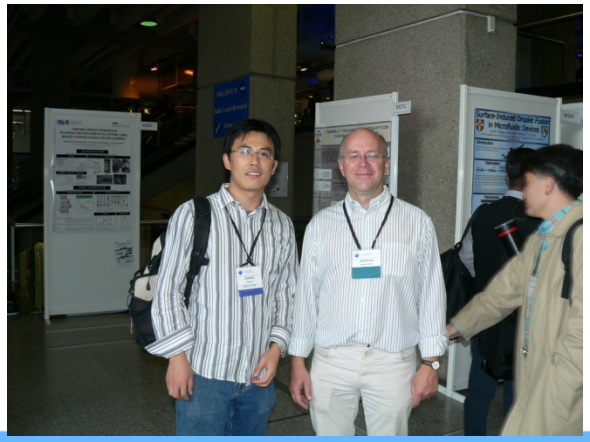
November 21st, 2014

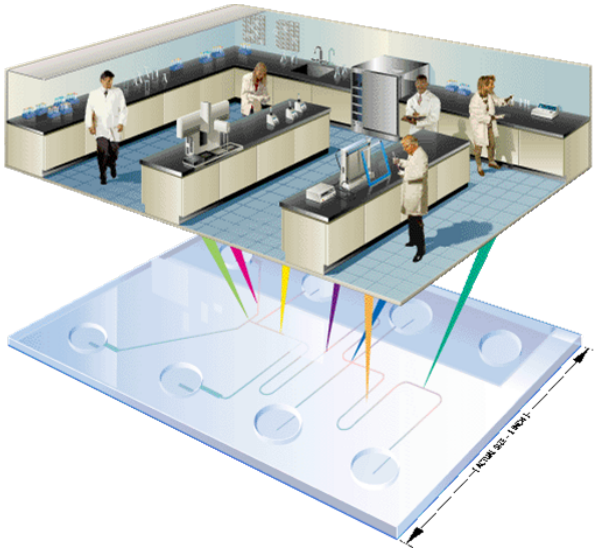
# Outline

- Microfluidic lab-on-a-chip
- Same-single-cell Analysis
- Hybrid microfluidic devices for infectious disease diagnosis
  - Integrated with aptamer-functionalized nanosensors for one-step pathogen detection
  - Integrated with DNA amplification for high-sensitivity meningitis diagnosis
- Conclusion

# What is microfluidic Lab-on-a-chip?

- Also called **micro total analysis system (μTAS)**
- Miniaturized system
- The major concept of a lab-on-a-chip system: **Integration**.
  - Dr. Manz, A.





# History

- Miniaturized system:
  - fast analysis, small reagent consumption, integration
  - 1979, miniaturized GC column coupled with TCD (thermal conductivity detector) on Si

**(Terry, S.C., et al., IEEE Trans. Electron. Devices, 1979, 26, 1880)**

  - 1992, Manz et al., the first demonstration of liquid-based miniaturized chemical analysis system
    - On a glass chip
    - **CZE (capillary zone electrophoresis)**
    - **EOF (electroosmotic flow)**

**(Manz, A., et al., J. Chromatogr. 1992, 593, 253.)**

# A Science paper

- D. Jed Harrison et al.
- Glass chip

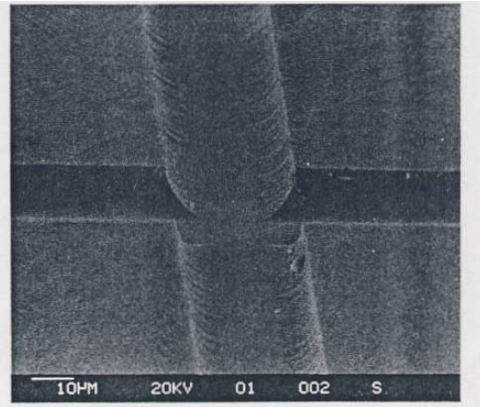
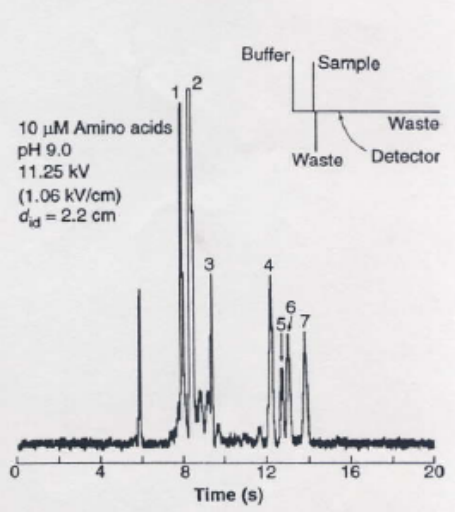


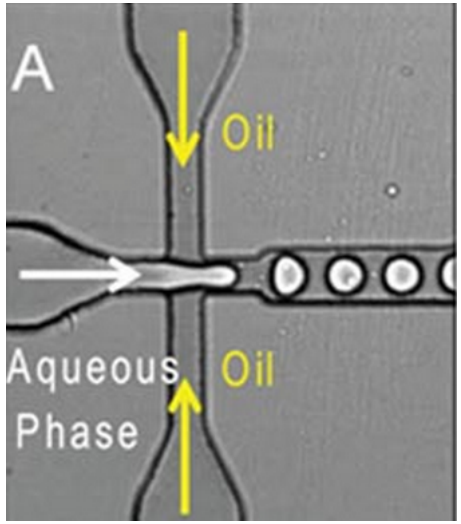

**Fig. 1.** Electron micrograph of capillary channels etched into Corning 7740 glass to a depth of 10 μm.




(Science, 1993, 261, 895)

# Why microfluidics?

- Miniaturized
  - Consume less space
  - Less reagent consumption
- Less sample needed.
  - Important for bioanalysis.
- Low cost
- High performance in CE separation
- Fast assay
- Integration
- high throughput
- Portable



# Glass micromachining

- Microfabrication
- Photolithography

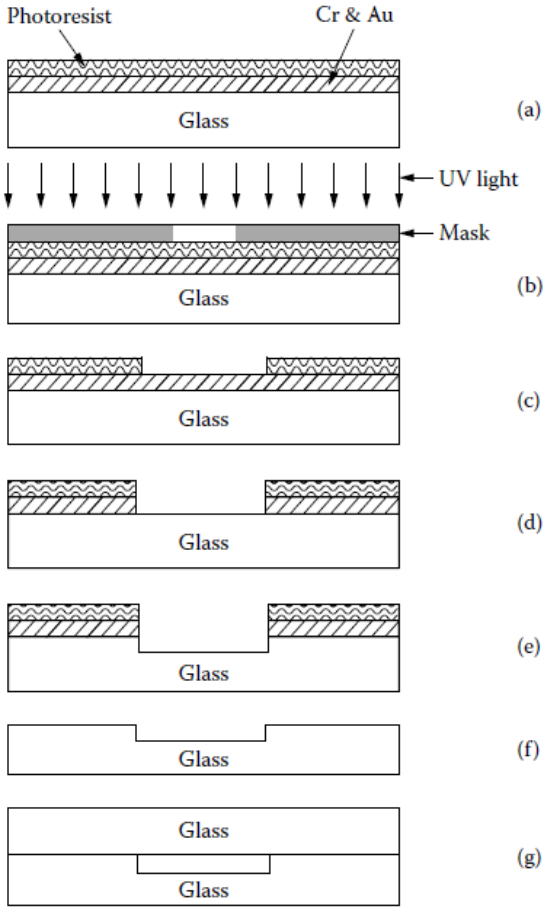
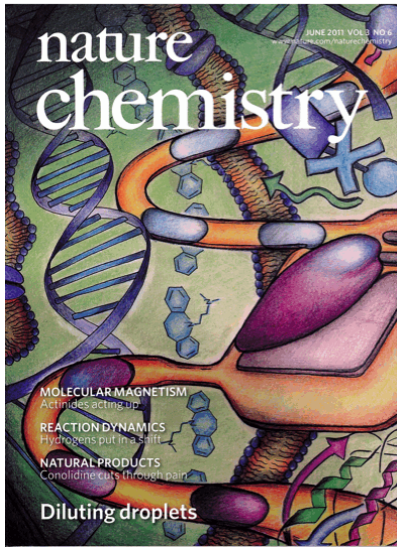
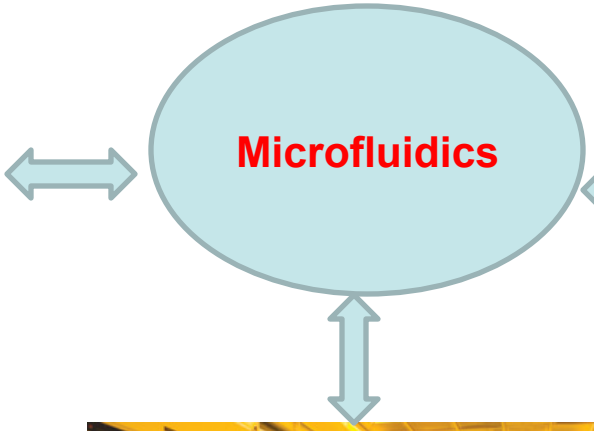



**FIGURE 2.3** Sequence for fabrication of the glass microfluidic chip. (a) Cr and Au masked glass plate coated with photoresist; (b) sample exposed to UV light through a photomask; (c) photoresist developed; (d) exposed metal mask etched; (e) exposed glass etched; (f) resist and metal stripped; (g) glass cover plate bonded to form sealed capillary [102]. Reprinted with permission from American Chemical Society.

# Microfluidic lab-on-a-chip

- Multidisciplinary:
  - Engineering, chemistry, life science



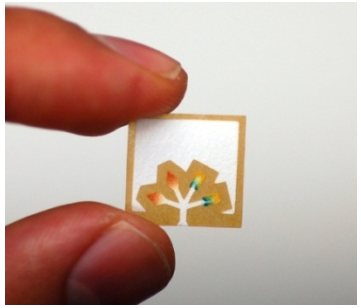




# Microfluidic Applications

- Broad applications, especially biomedical application.
- Cellular analysis


**Xiujun Li;** Paul C.H. Li; ***Anal. Chem***. **2005**, *77*, 4315-4322

- Protein analysis
- Low-cost medical diagnosis

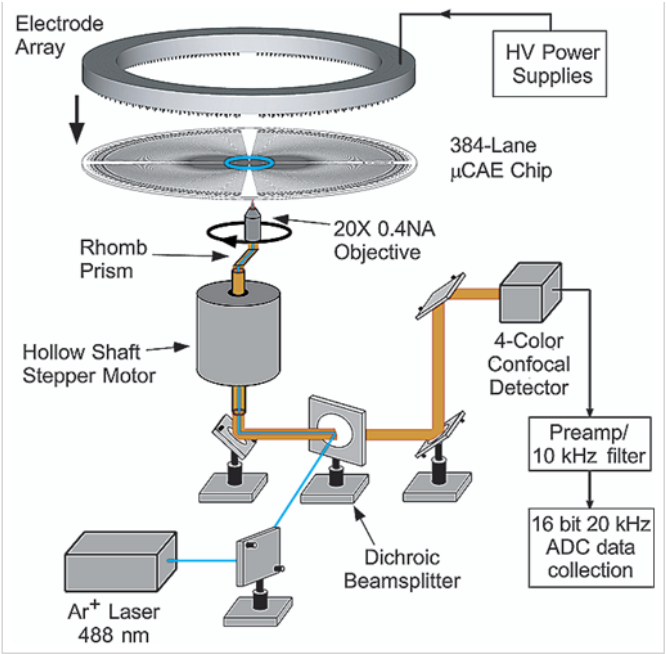


**George Whitesides**
**Harvard University**

# Applications: Genetic assay

**Richard Mathies**
**UC Berkeley**

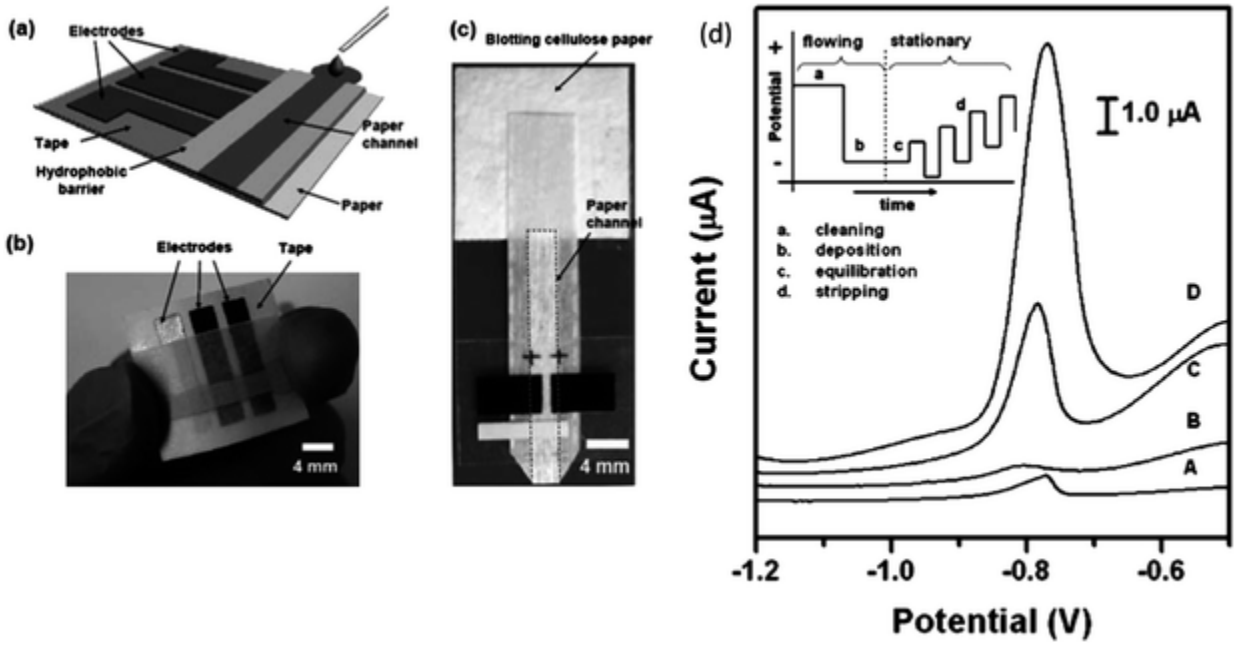
- Rotary fluorescence scanner
- 384 channels!
- High throughput.




**Emrich, C.A. et al, Anal. Chem. 2002, 74, 5076–5083.**

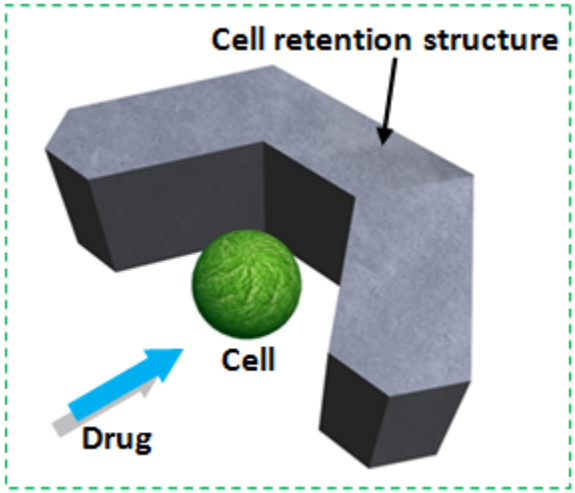
# Application-Environmental analysis

- Heavy metal analysis




**Nie, Z.H., et al., Lab Chip, 2010, 10, 477–483**

# I. Same-single-cell analysis (SASCA) for the study of drug efflux modulation of multidrug resistant cancer cells

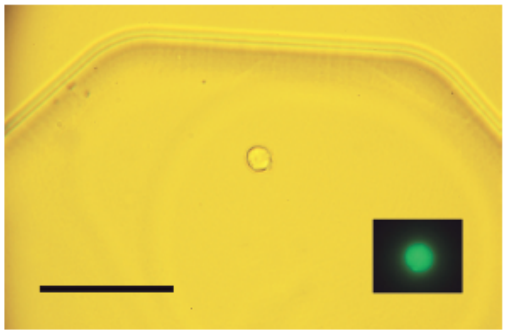



news

## SASCA tackles single drug-resistant cancer cells

uliar observation: in colleagues at Simon Canada) had designed e with 3D flow con- ure and manipulate *Chem.* **2004**, *76*, doing e investi- the single o spit out ker they using the rease. prompted think ld turn nom- lux, into that In their **08**, *80*, Li, Xiujun Li, and Victor Ling describe how they use the microfluidic device to measure drug

precious few cells are at hand. To circumvent the sample size issue, a single-cell microfluidic analysis technique, called different-single-cell analysis (DISCA), has been developed to work with a limited number of cells.



A captured, live, single cell stained with fluorescein diacetate sits in the observation chamber of a microfluidic device. Scale bar: 50 μm.

same cell, but this time, the efflux is measured in the presence of an inhibitor. "We see the results clearly," says Paul Li, who notes that much of the work was done by his graduate student, Xiujun Li. "If the cell has low multidrug resistance, then we see a little bit of a reversal with the inhibitor. In the case of high multidrug resistance of a cell, we see a much better and greater reversal effect" with the inhibitor.

Once they were convinced that SASCA worked well, the investigators tested two compounds from traditional Chinese herbal medicine for the ability to inhibit drug efflux. Isoliquiritigenin is derived from Chinese licorice; by the SASCA method, it didn't demonstrate any inhibition of drug efflux.

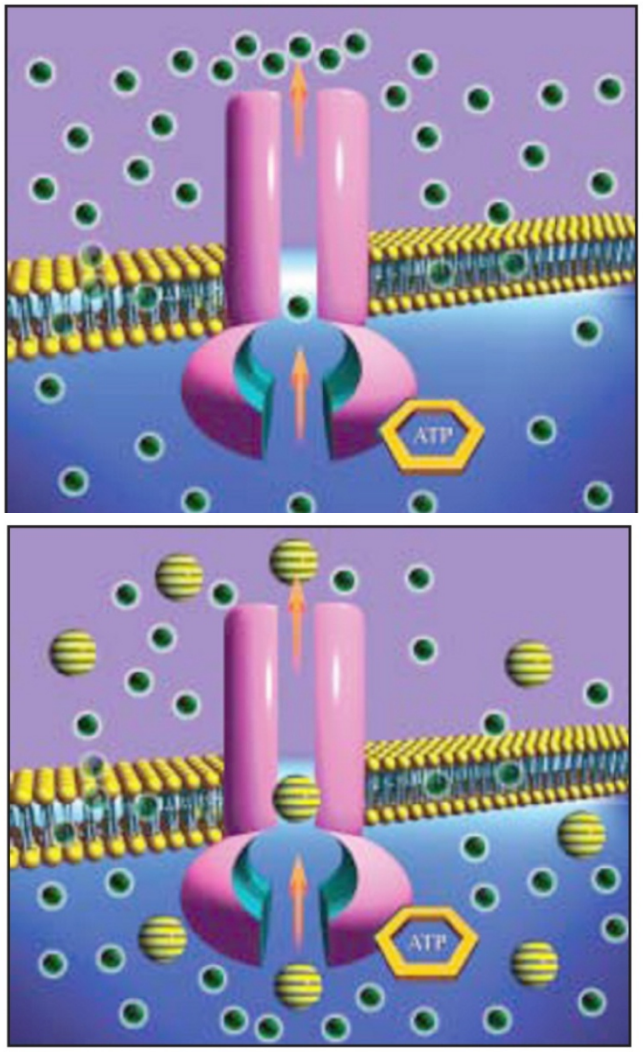
**Anal. Chem. 2008, 80, 3951.**



•CEM, Human acute leukemia cell line.

# Multidrug resistance (MDR) & drug efflux

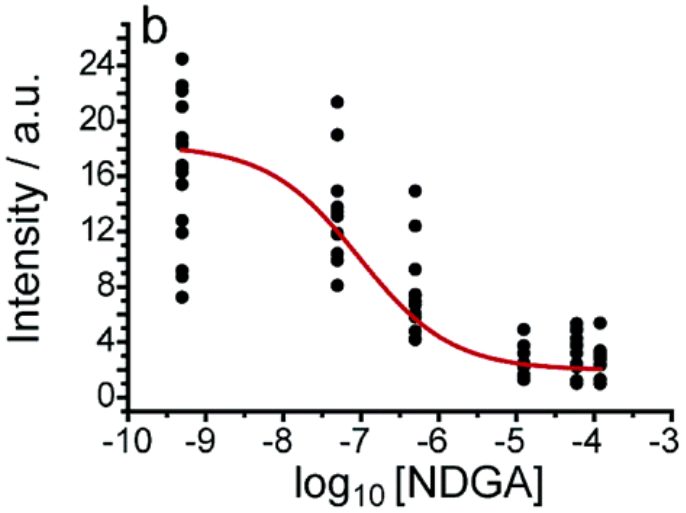
- MDR is a major obstacle to successful cancer chemotherapy.[1]
- MDR causes low drug retention in the cell.
- MDR is mediated by P-glycoprotein (Pgp) pumps, causing drug efflux.
- Pgp, membrane-bound drug efflux pump, actively transports drugs out of the cancer cell and causes lower drug retention inside the cell.
- Drug efflux can be reversed using MDR modulator.




1: E. Marthine *et al. Gene Therapy*, 2000, 7, 1224

# Different-single-cell Analysis (DISCA)

- When a control experiments in single-cell analysis is needed, usually a different-single-cell approach is adopted.
- Carlo *et al.* compared the different inhibition of intracellular carboxylesterases of HeLa cells at different concentrations of inhibitor.[1]

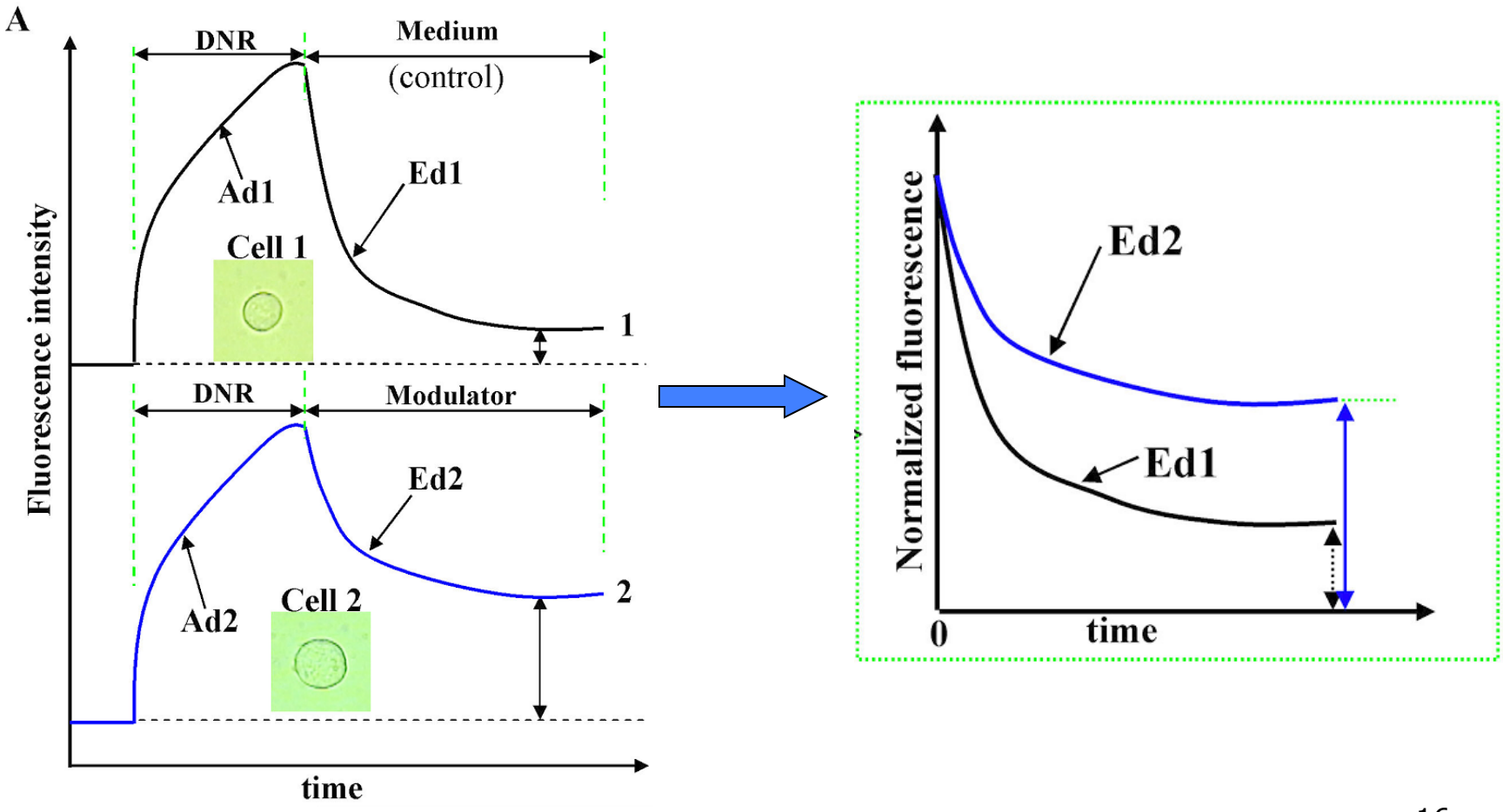





1. Carlo, DD. *et al*. *Anal. Chem*. **2006**, *78*, 4925-4930

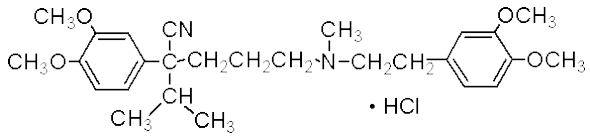
# Different-single-cell analysis (DISCA)

- Different-cell control

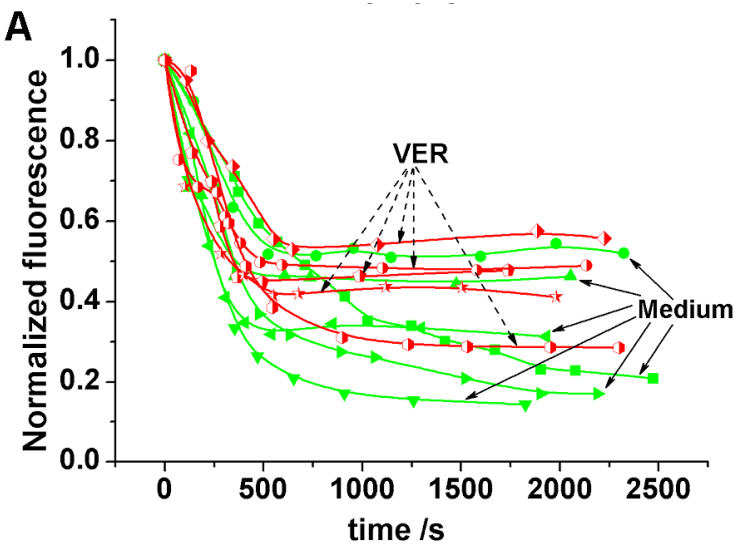


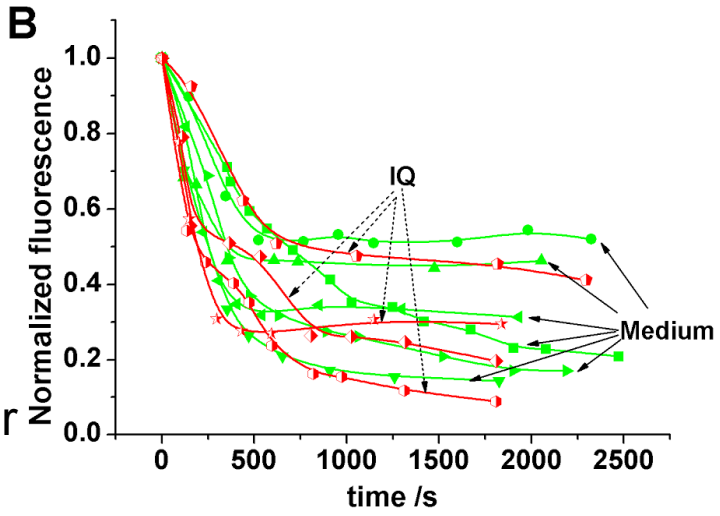
# Drug efflux by DISCA

- A lot of variations were observed among different cells
- **Cellular heterogeneity may obscure positive drug effects**




Verapamil (Ver), a well-known MDR modulator



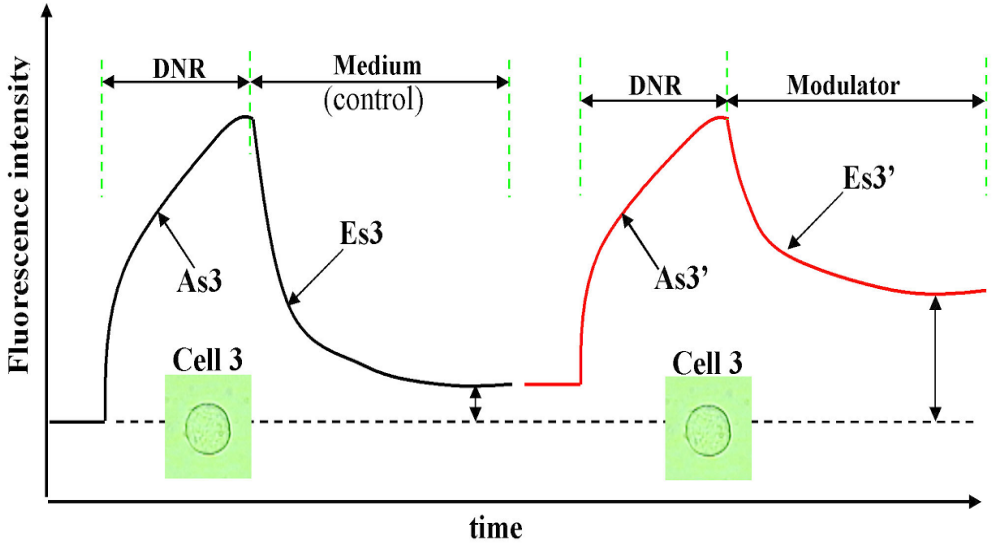



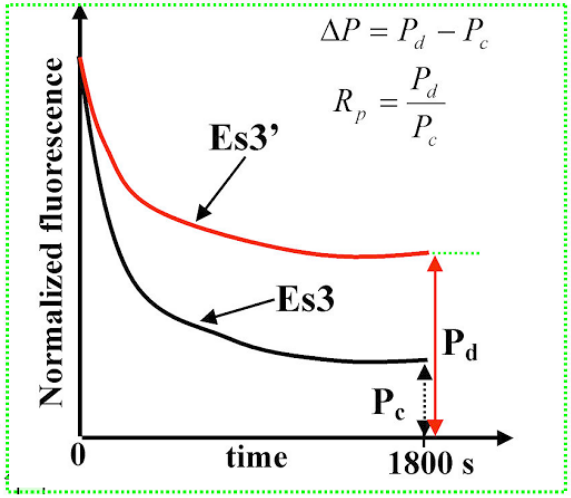



**XiuJun Li**, Victor Ling, Paul CH Li, ***Anal. Chem***. 2008, *80*, 4095-4102.

# Same-single-cell analysis (SACSA)

- Same-cell control

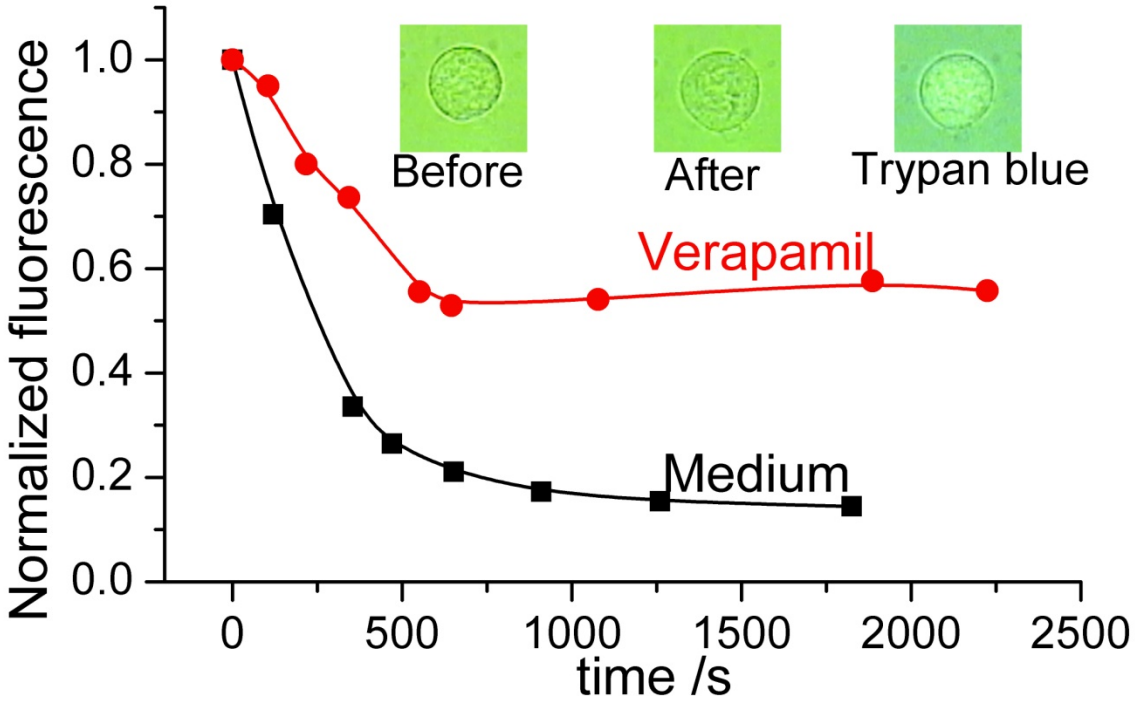


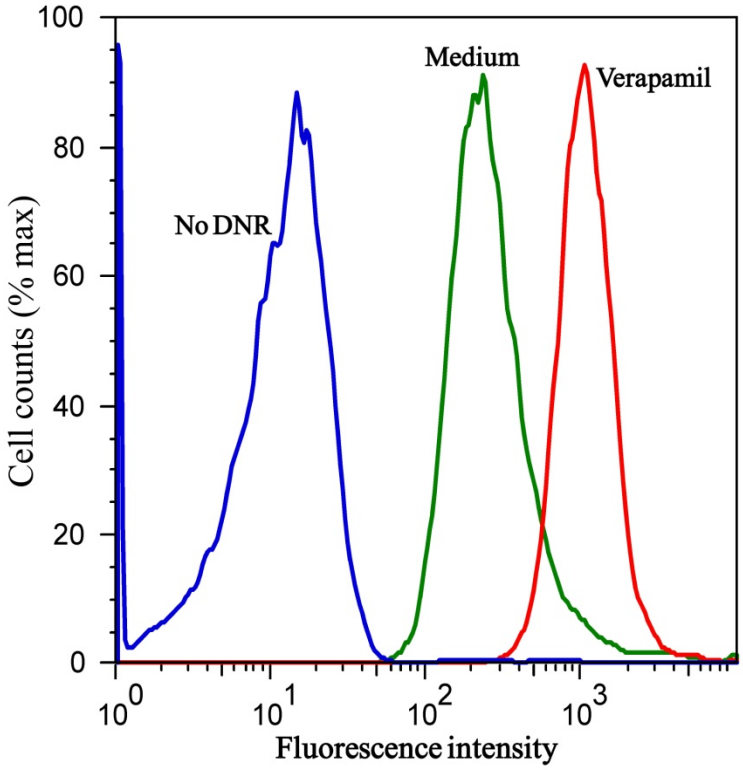






**XiuJun Li**, Victor Ling, Paul CH Li, ***Anal. Chem***. 2008, *80*, 4095-4102.

# Drug efflux modulation by Verapamil by SASCA & flow cytometry



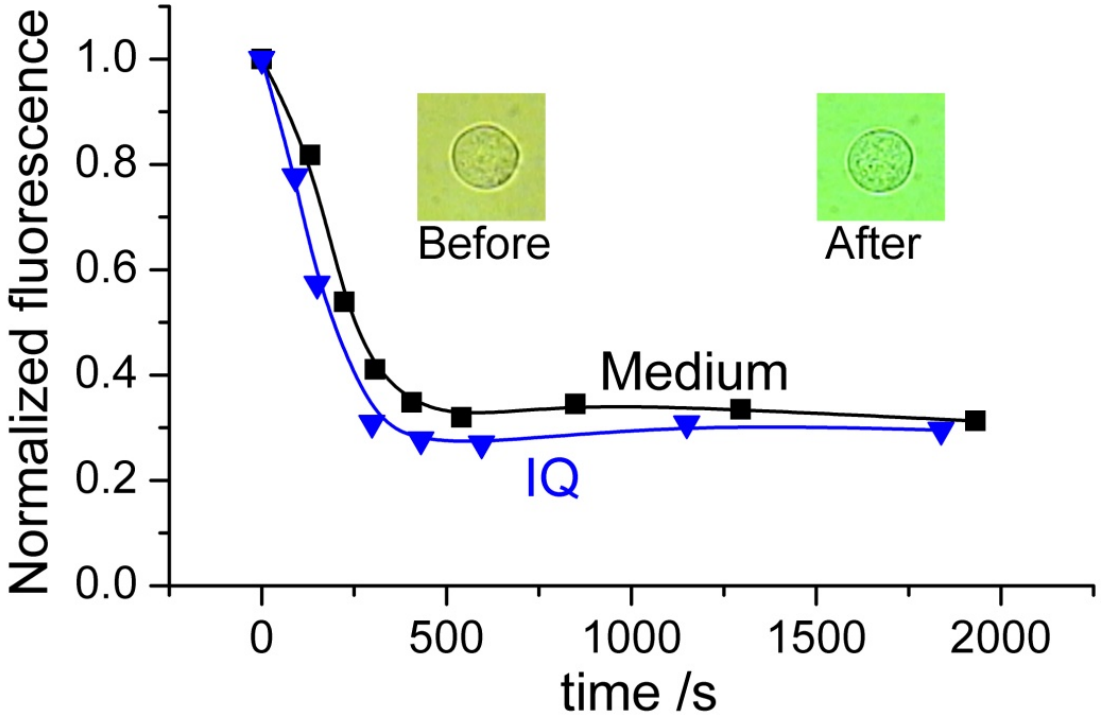






**XiuJun Li**, Victor Ling, Paul CH Li, ***Anal. Chem***. 2008, *80*, 4095-4102.

# IQ effect on drug efflux by SASCA

- IQ doesn't have MDR reversal effect.



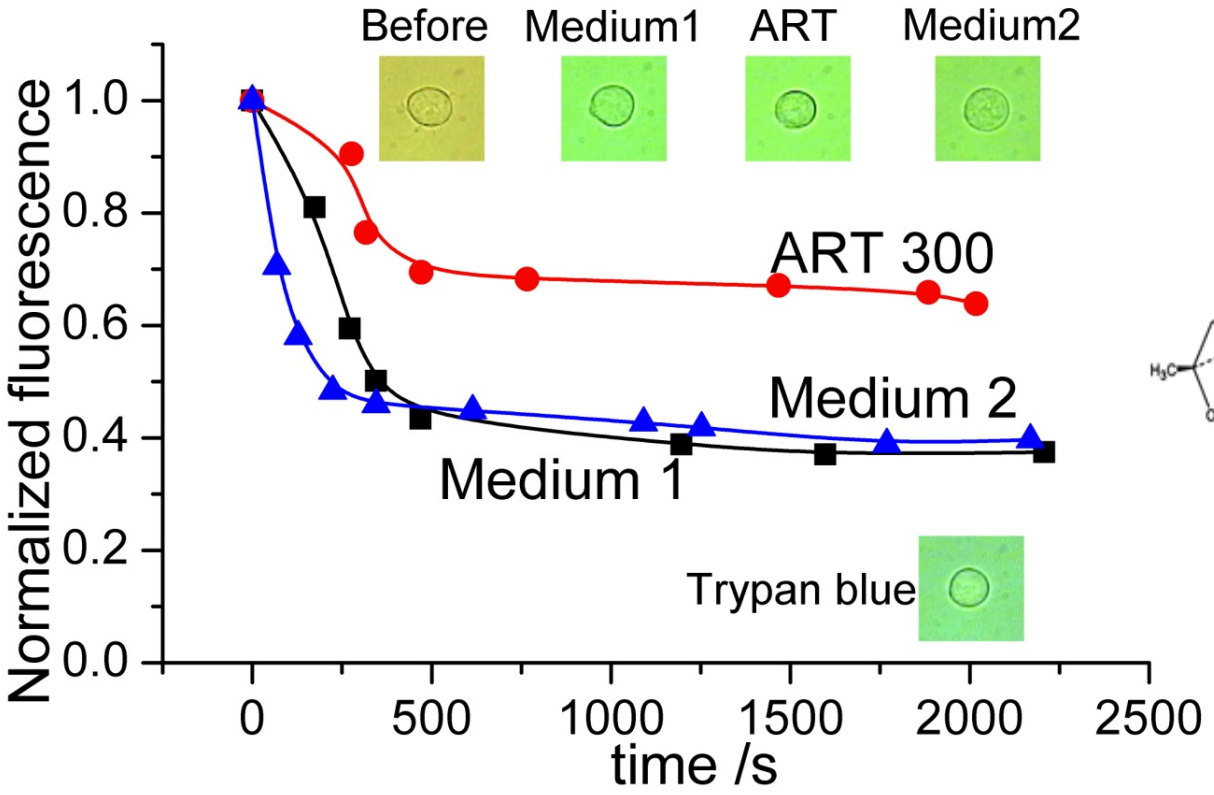

•IQ (Isoliquiritigenin), a licorice ingredient.

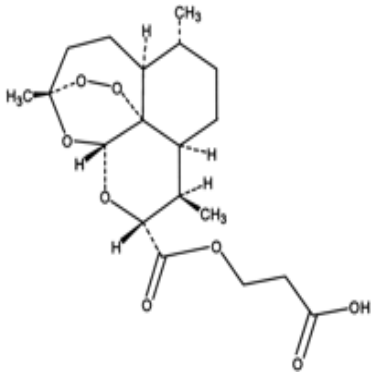


**XiuJun Li**, Victor Ling, Paul CH Li, ***Anal. Chem***. 2008, *80*, 4095-4102.

# Artesunate effect on drug efflux by SASCA

- Artesunate (ART), a herbal ingredient
- Two times control.



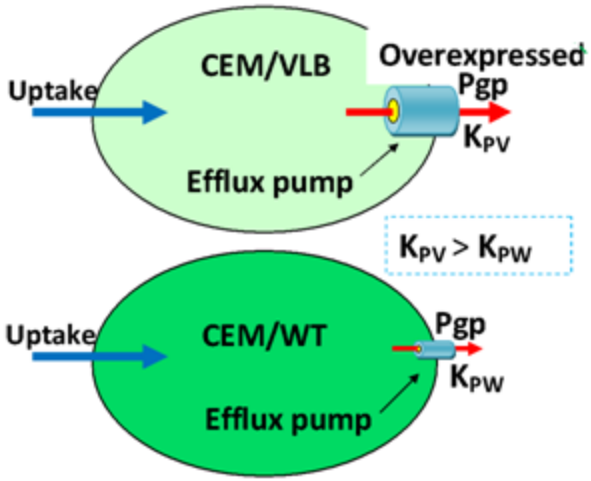



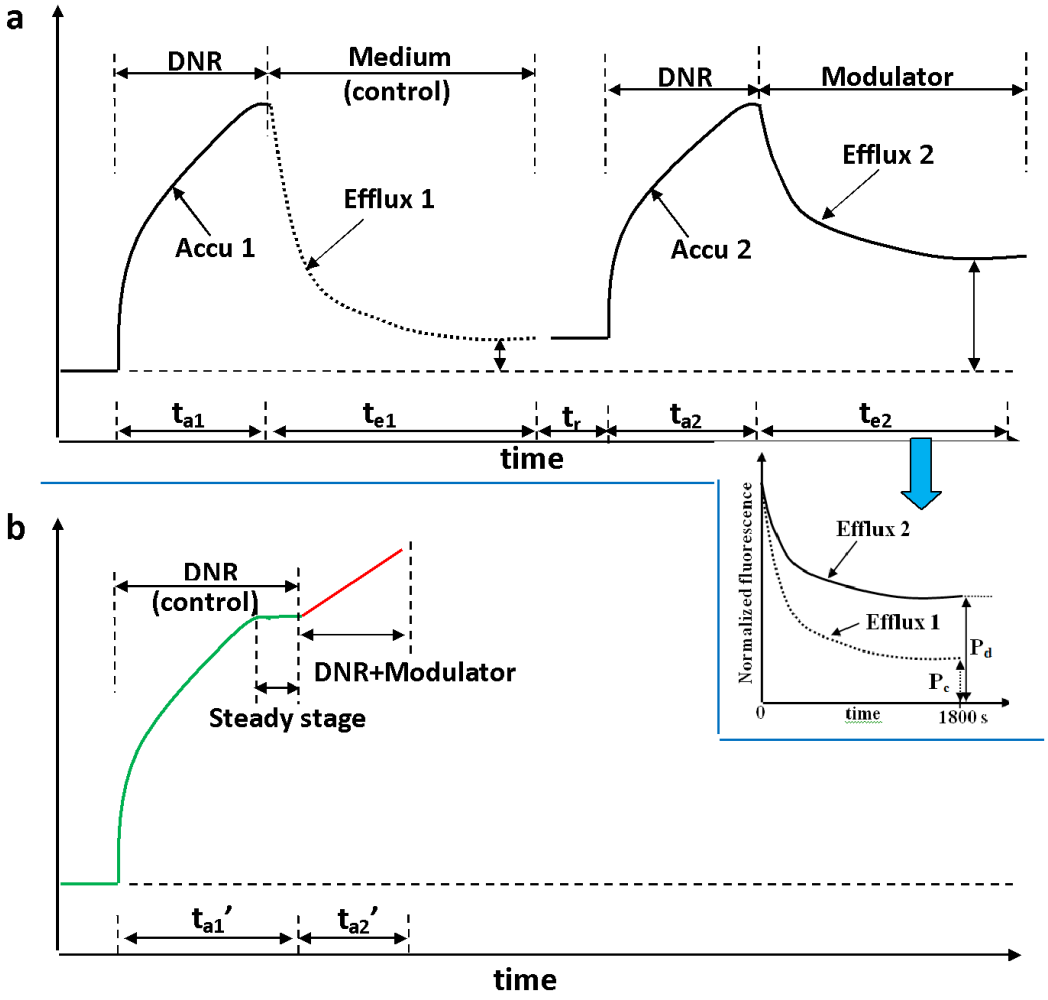


**XiuJun Li**, Victor Ling, Paul CH Li, ***Anal. Chem***. 2008, *80*, 4095-4102.

# Improvement: a simpler & faster SASCA-A

- Accumulation stage.
- Simpler
- Faster
- More ‘identical’ control.



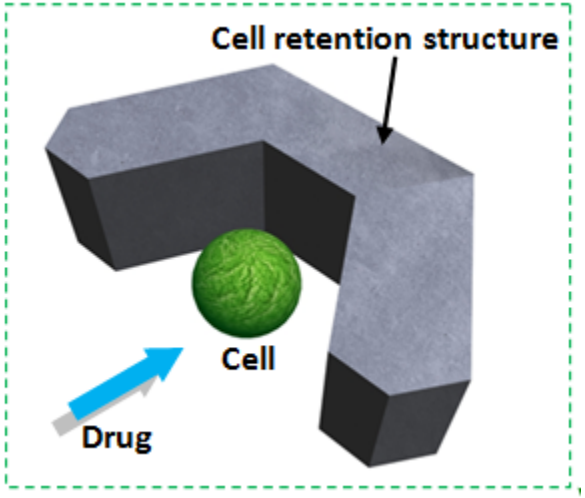






**Xiujun Li**, Y. Chen, P. C.H. Li ***Lab Chip*** , **2011**, 11, 1378-1384.

# Conclusions –I.

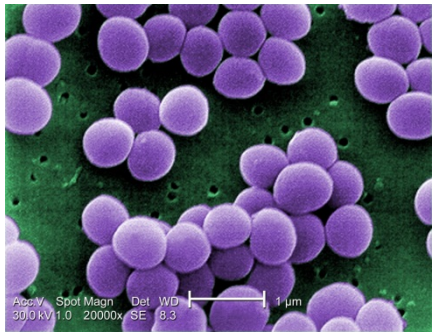
- A new concept of SASCA has been put forward to address cellular variations in single cell study, by using the same cell as its control. Its advantages have been demonstrated in the drug efflux study of MDR cancer cells.



# II. Hybrid microfluidic devices for infectious disease diagnosis

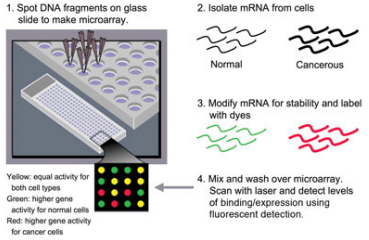
# Infectious diseases


- Microorganism Pathogens
  - *E. Coli*, *Salmonella*, *S. aureus* and …
- Often cause serious global health concerns and economic loss
  - *HIV, TB, SARS, HBV, Malaria, Ebola…*
  - Often happened in high-poverty locations.
- Food-borne pathogen
- Meningitis



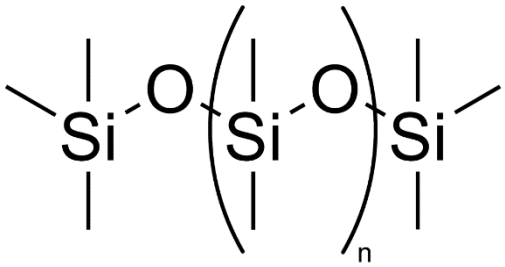
# Pathogen Detection Techniques

- **Gram stain**, low sensitivity, not reliable
- **Bacterial culture**, time-consuming.
- **DNA-based testing**, e.g. DNA microarray, RT-PCR
  - **RT-PCR**, ~$60,000
  - Requires complicated DNA extraction steps.




- **Immunoassay**
  - Expensive. Enzyme can lose activity at room temperature quickly.
- Expensive, bulky equipment, complicated procedures → not suitable for on-site detection
- **Goal:** develop low-cost microfluidic devices for simple infectious disease diagnosis, in low-resource settings.

# Why PDMS?

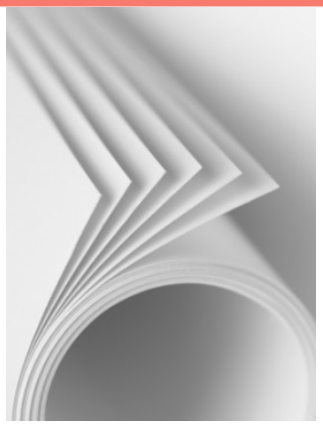



- Inexpensive
- Ease of fabrication procedures
- Good optical properties
- $O_2$ permeable

Why not PDMS?

- Surface properties, e.g. hydrophobic
- Autofluorescence
- Surface treatment needed to immobilize sensors or probes

# Why paper?

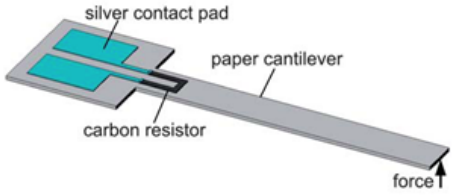


- Low-cost
- Simple fabrication procedures
- Good stackability
- High surface-to-volume ratio, → Increase reaction kinetics
- 3D storage matrix

Why not Paper?

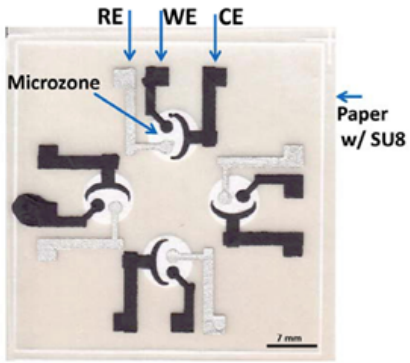
- Low performance in flow delivery & control
- Not transparent

A




Liu, X., et al. *Lab Chip*, 11(13): 2189-2196.

B




Li, X. J.; et al,. *Proc. Micro Total Analysis*

# Hybrid…


**Hybrid Rice**

# II. Hybrid microfluidic devices

- Integrated with aptamer-functionalized graphene oxide (GO) nanosensors for simple one-step food-borne pathogen detection

Dr. Peng Zuo

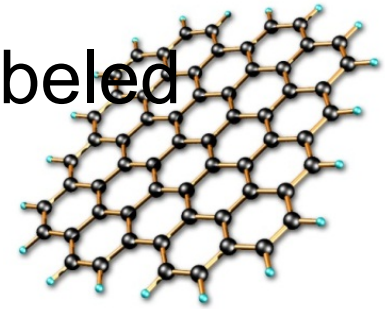
# Aptamer-based detection

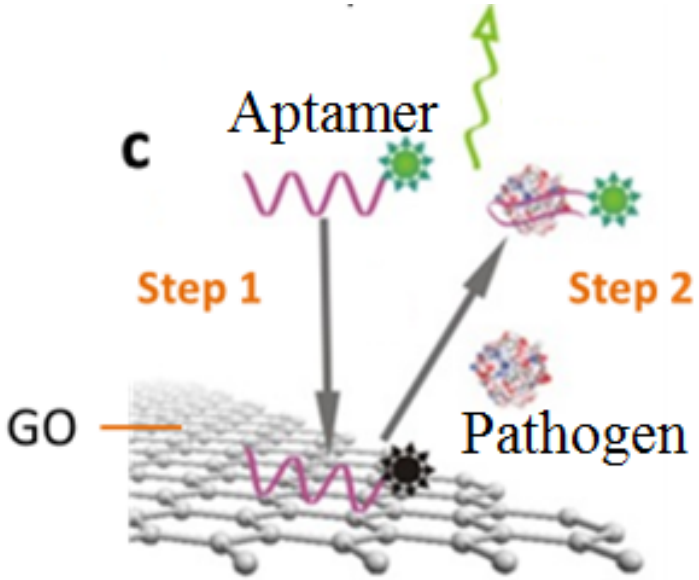
- Aptamers, oligonucleic acids that bind to a specific target
- Directly target to microorganisms
- Can be easily synthesized
- More stable than antibodies/enzyme

| Aptamer | Sequences (5'-3') |
|---|---|
| *L. acidophilus* (FALA) | cy3- ATC CGT CAC ACC TGC TCT ACG GCG CTC CCA ACA GGC CTC TCC TTA CGG CAT ATT ATG GTG TTG GCT CCC GTA T |
| *S. aureus* (FASA) | cy3- GCA ATG GTA CGG TAC TTC CTC GGC ACG TTC TCA GTA GCG CTC GCT GGT CAT CCC ACA GCT ACG TCA AAA GTG CAC GCT ACT TTG CTA A |
| *S. enterica* (FASE) | cy3-TAT GGC GGC GTC ACC CGA CGG GGA CTT GAC ATT ATG ACA G |

# Aptamer-functionalized GO biosensors

- Graphene oxide (GO), a 2D nanomaterial
- Unique properties in quenching fluo-labeled DNA oligoes.
- A one-step “Turn-on” mechanism

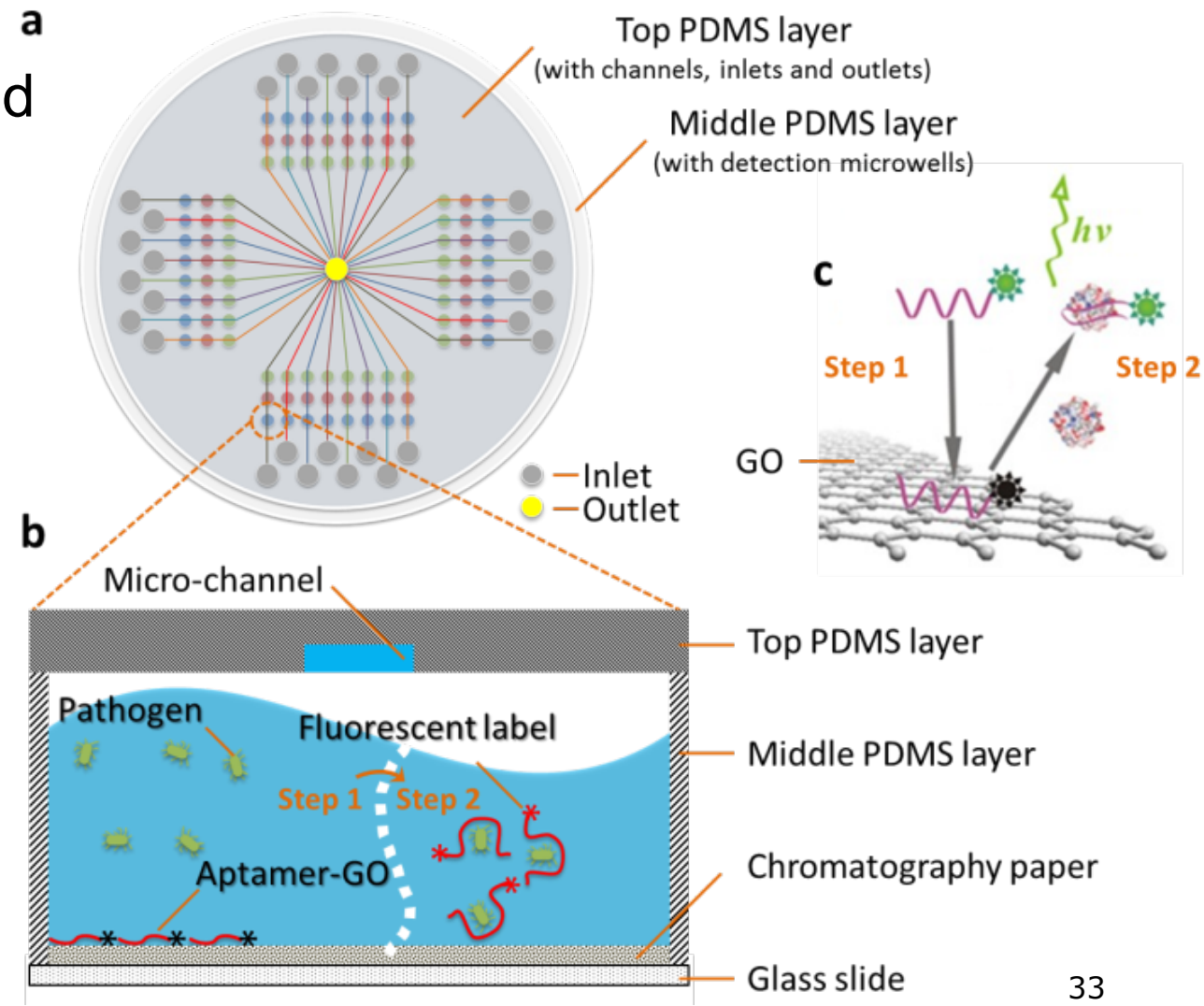





P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# A paper/PDMS hybrid microfluidic system integrated with aptamer-functionalized nanosensors

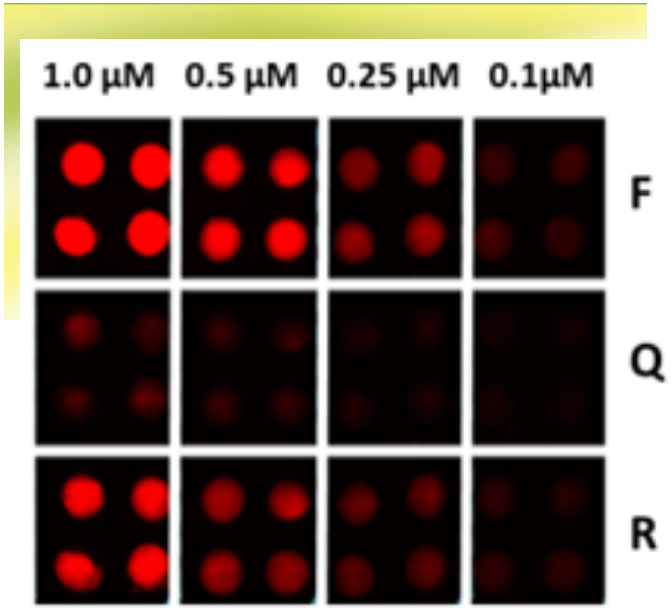
- Paper→ facilitated GO sensor immobilization
- Avoided complicated surface modification for nanosensors immobilization

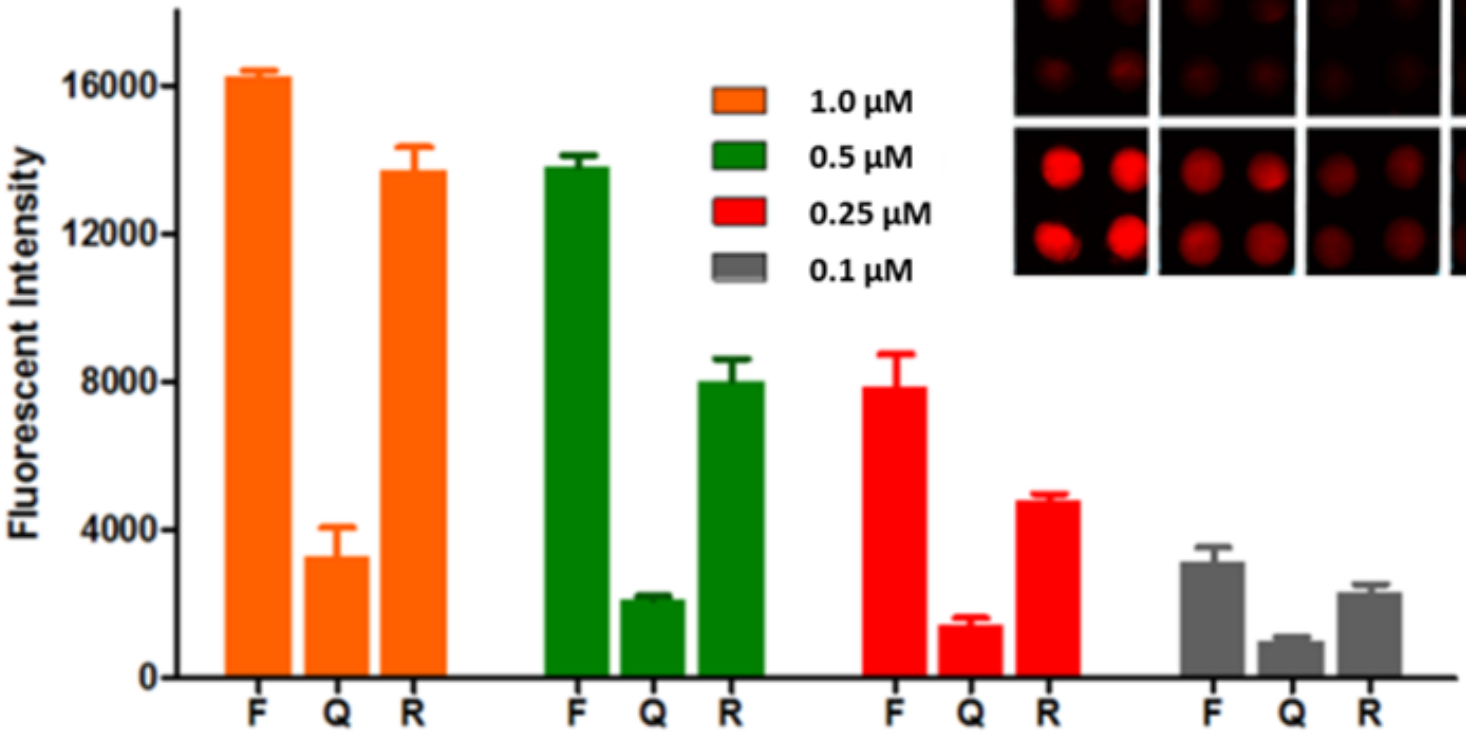



P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# Aptamer Concentration Optimization

- *L. acidophilus* as a model 'pathogen'



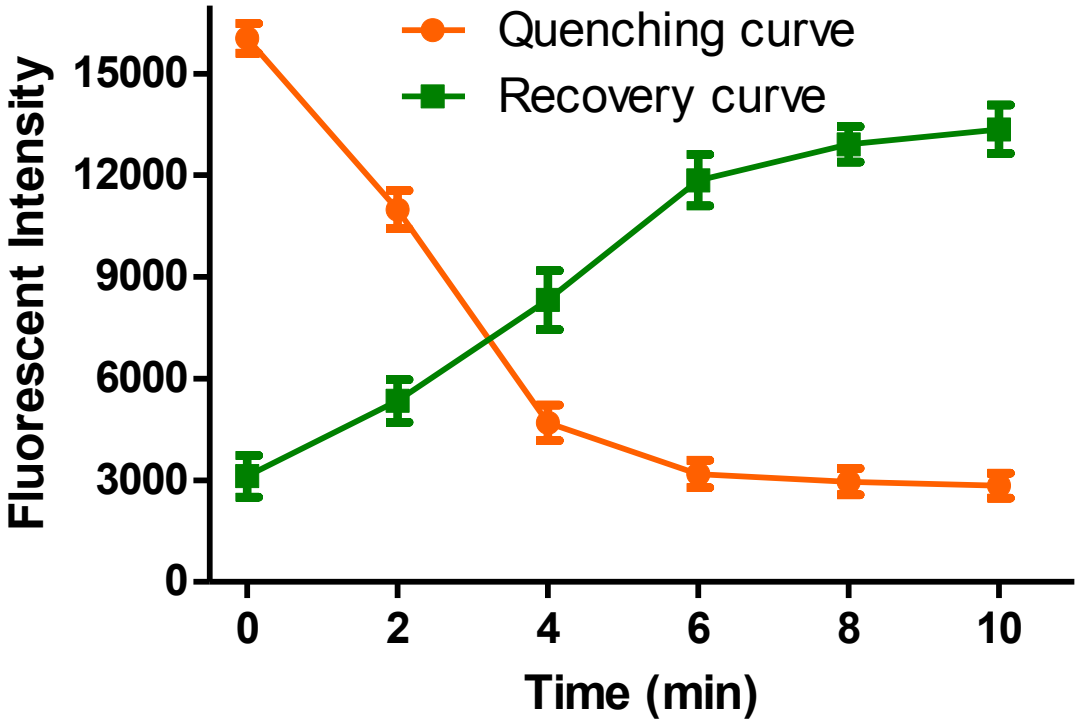






P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# Reaction Time Optimization

- Quenching time & Recovery time

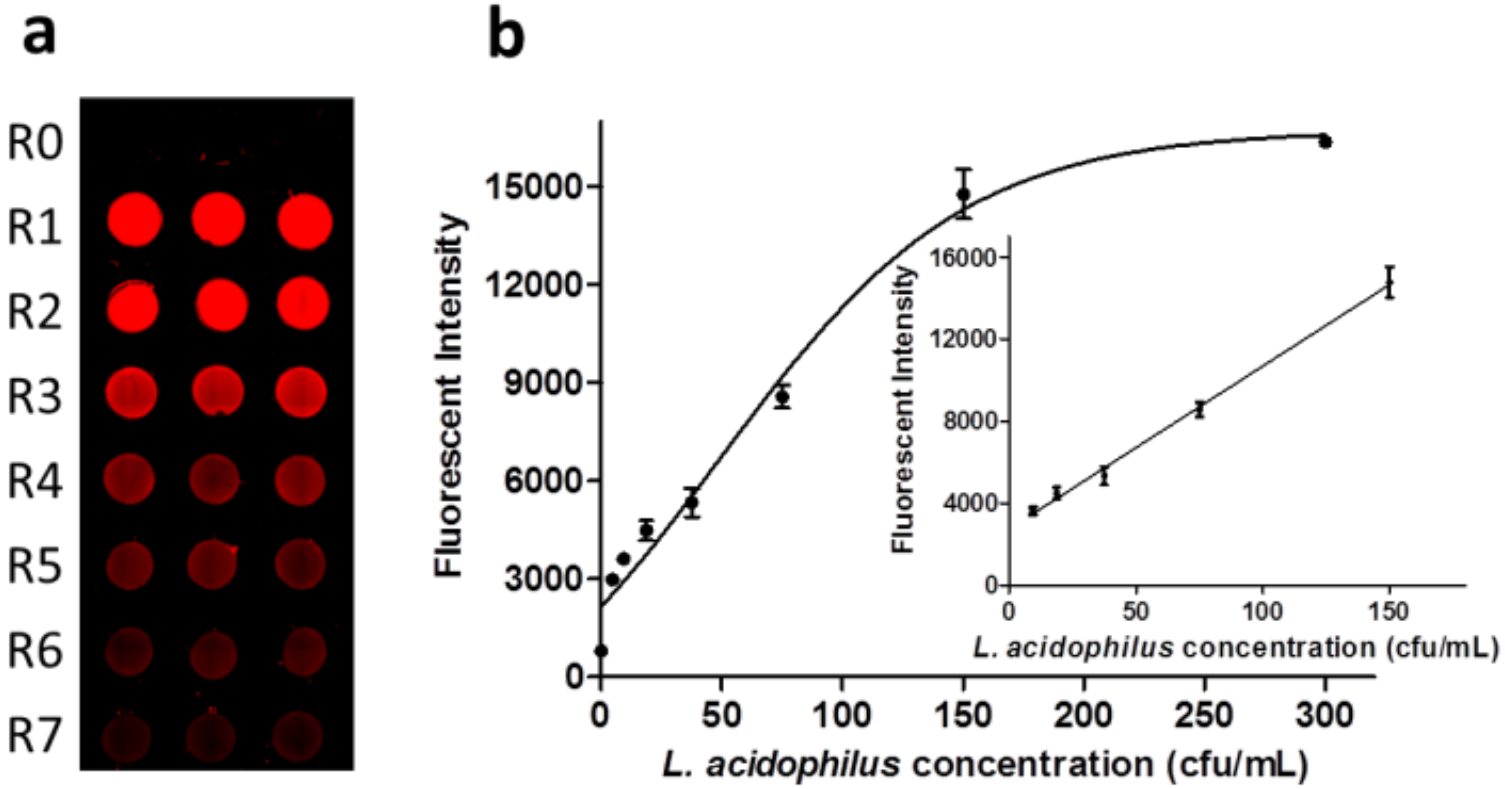





P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# Calibration Curve

- LOD, ~11.0 cfu/mL



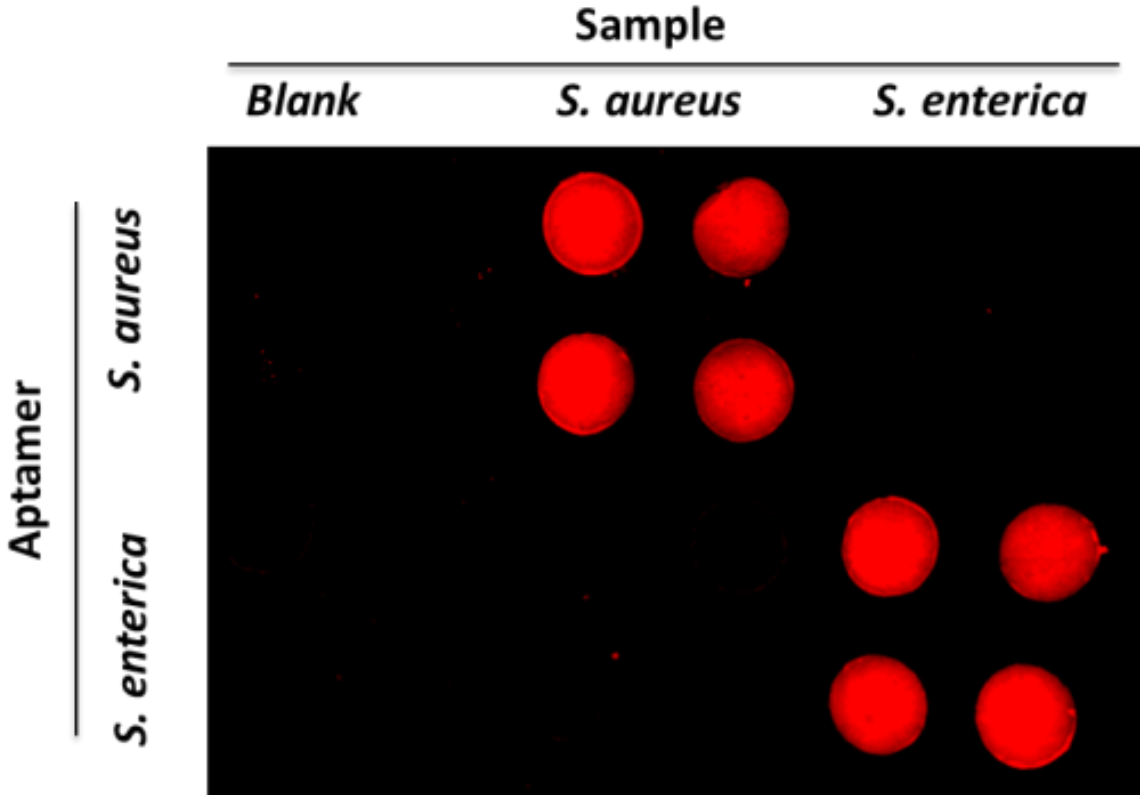



P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# One-Step pathogen detection

- Simultaneous detection of *S. aureus* & *S. enterica*

- Aptamer
  - Direct pathogen detection
  - Avoided cumbersome DNA preparation steps
- High simplicity
  - One-step
- Fast, 10 min



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# Sample Test

| Pathogen | Spiked cfu/mL | Average measured cfu/mL | Coefficient of Variation | Percent Recovery |
|---|---|---|---|---|
| S. enterica | 84.4 | 78.4 | 7.3% | 92.9% |
| | 168.8 | 162.7 | 5.5% | 96.4% |
| S. aureus | 50000.0 | 51668.4 | 7.3% | 103.3% |
| | 500000.0 | 539371.2 | 9.5% | 107.8% |



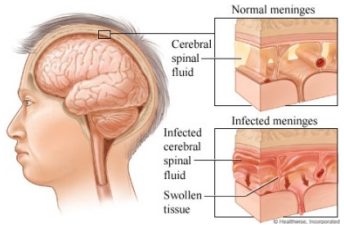
P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

# II. Hybrid microfluidic devices

- A versatile hybrid biochip integrated with DNA amplification for Instrument-free high-sensitivity Infectious Disease Diagnosis

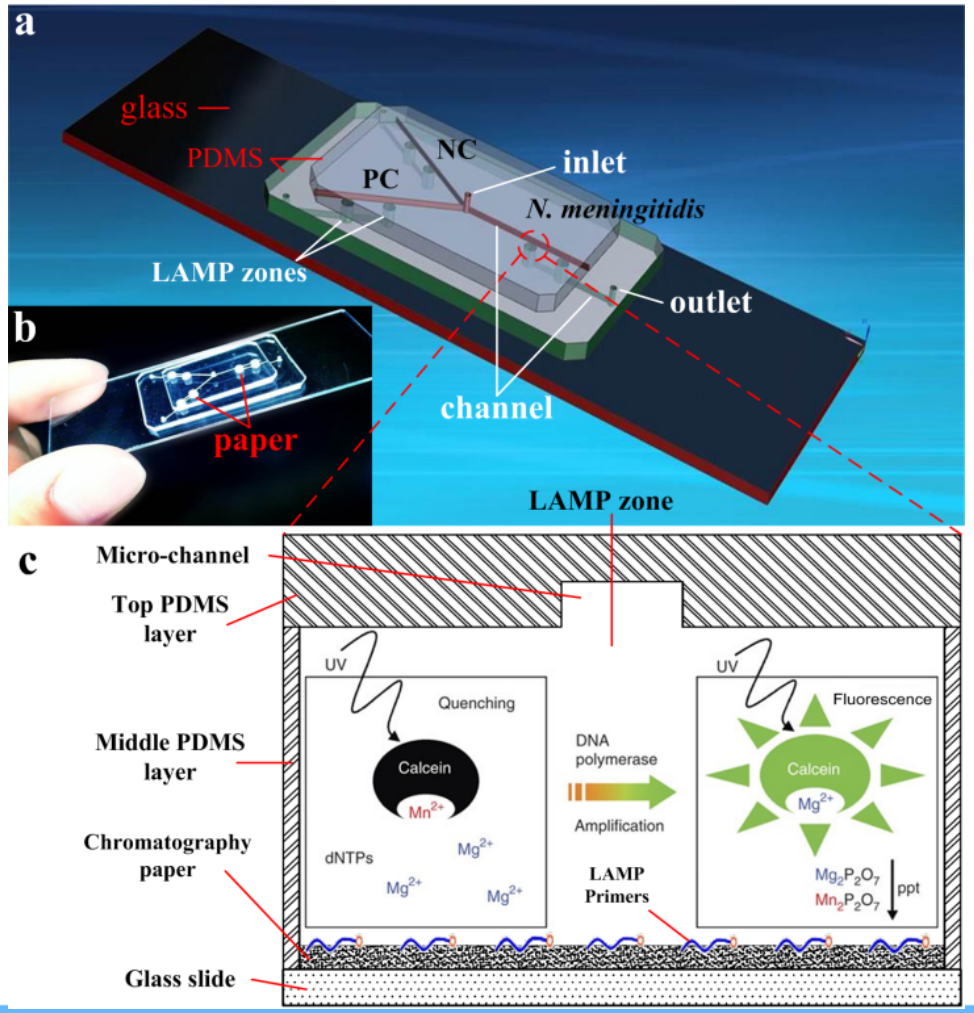
Maowei Dou

# Meningitis



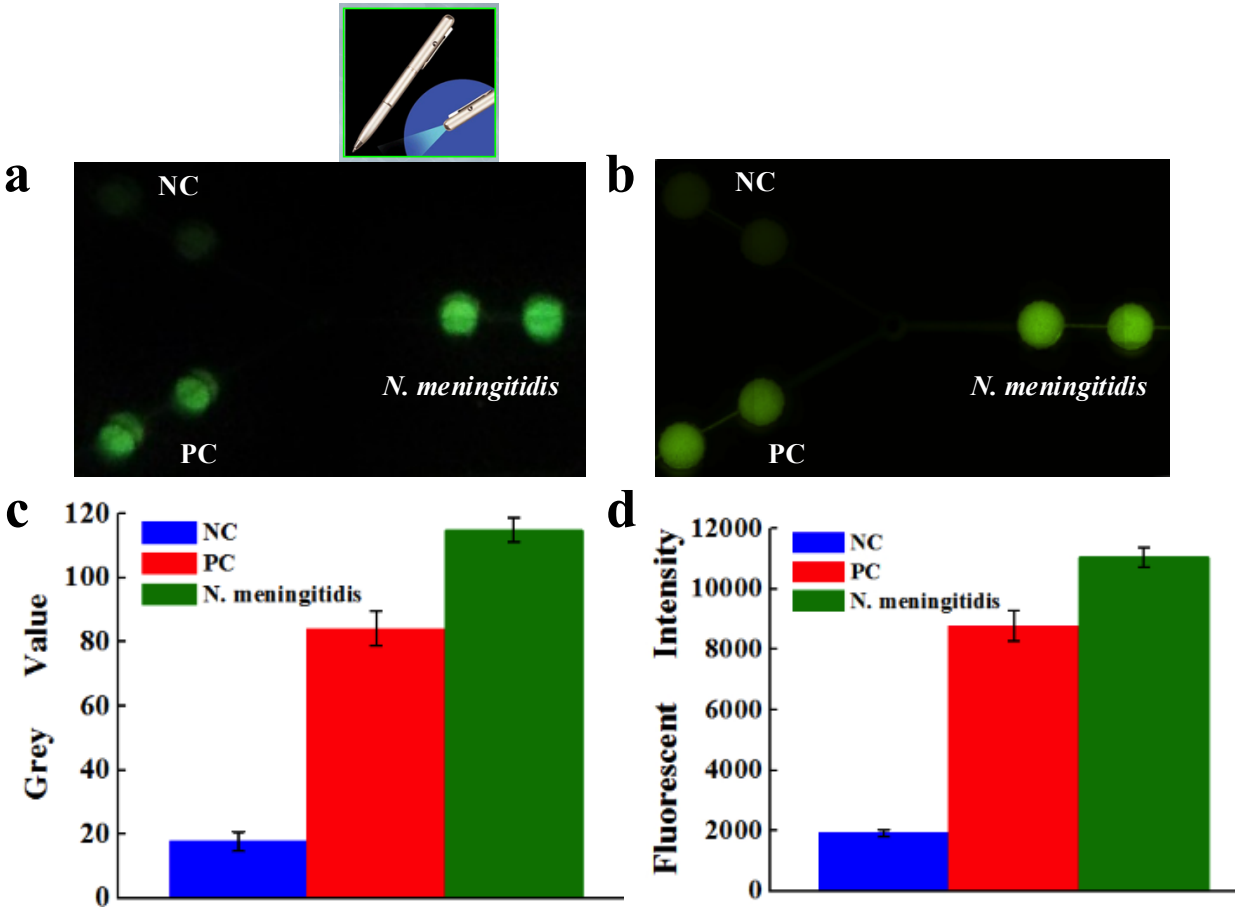

- Meningitis
  - Infection of the membrane of brain and spinal cord
  - High mortality (30-60%)
  - Fast-acting, 2 days
  - High morbidity
    - WHO "Worldwide, without epidemics one million cases of bacterial meningitis are estimated to occur and 200,000 of these die annually. "
  - High poverty regions

# A new Paper /PDMS hybrid microfluidic system integration with DNA amplification



# Instrument-free Detection

- Potable UV light pen
- Camera phone
- ImageJ
- *N. meningitidis*

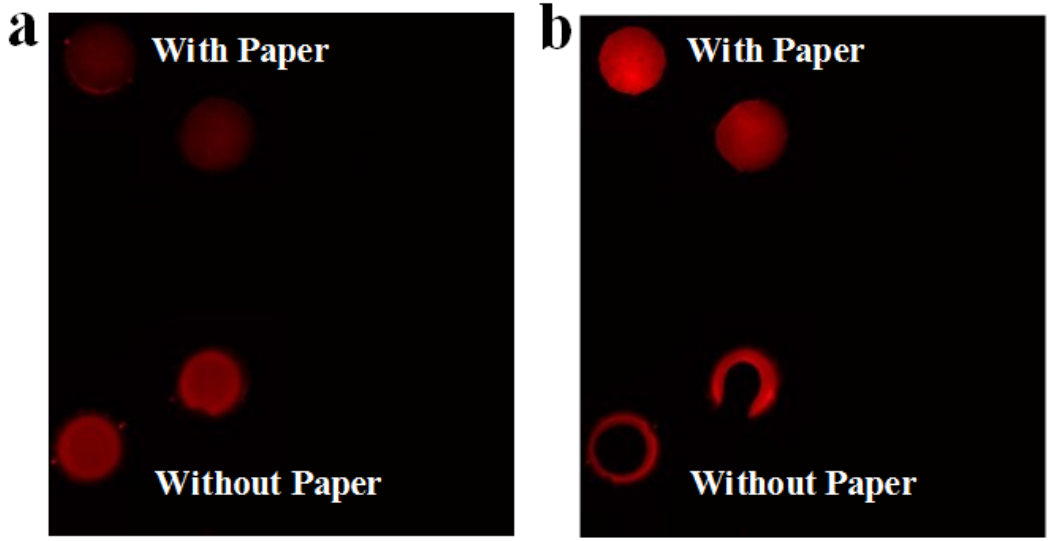


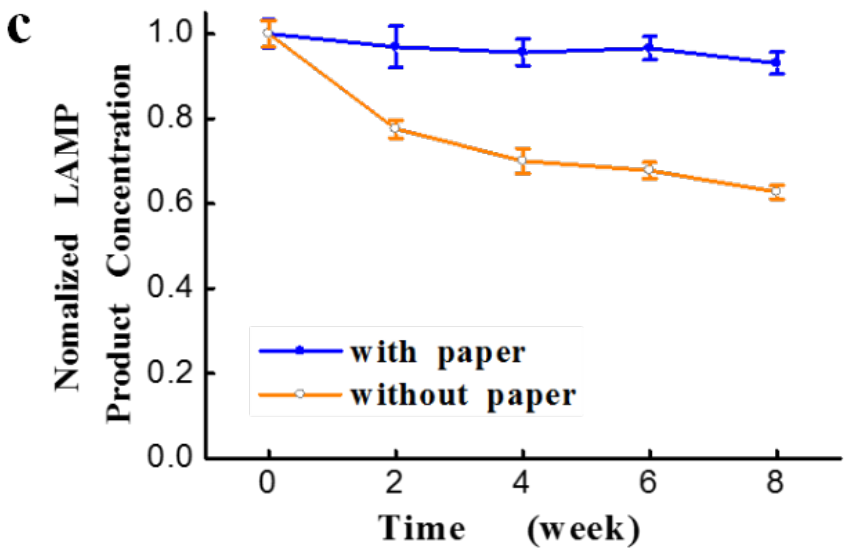



Dou, M, et al, *Anal. Chem*. 2014, 7978

# Paper?

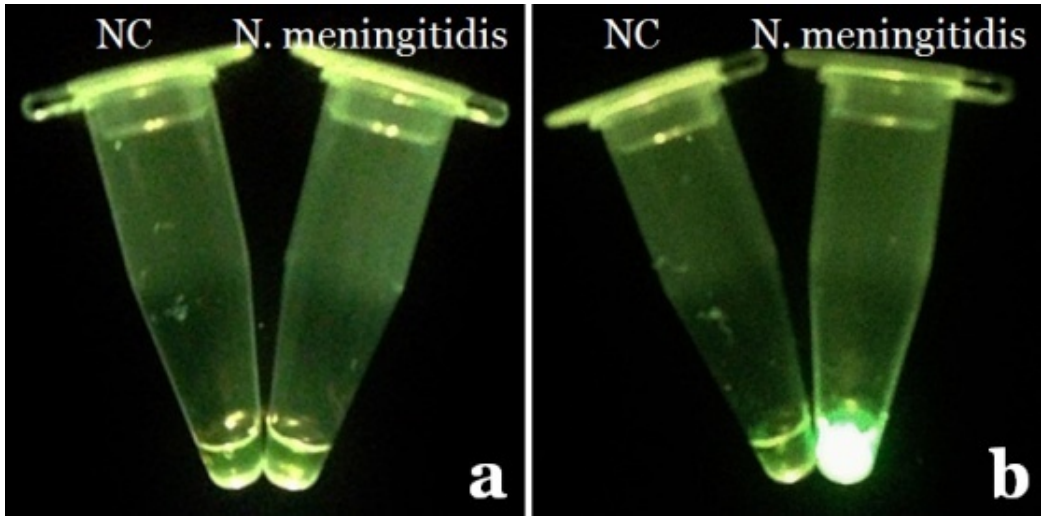
- 3D storage substrate
- Uniform primer distribution
- More stable performance

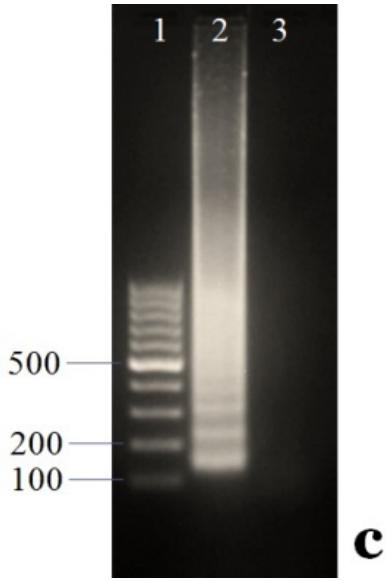


# Versatile Functions

- Amplicons can be readily extracted for confirmatory analysis



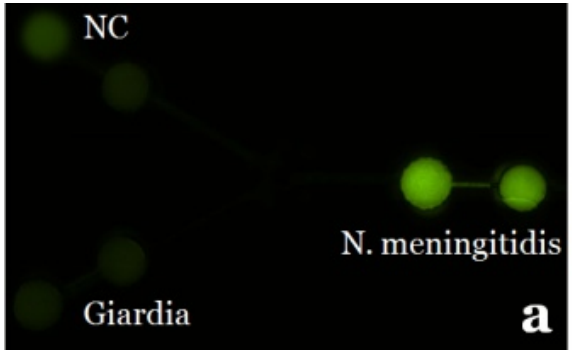



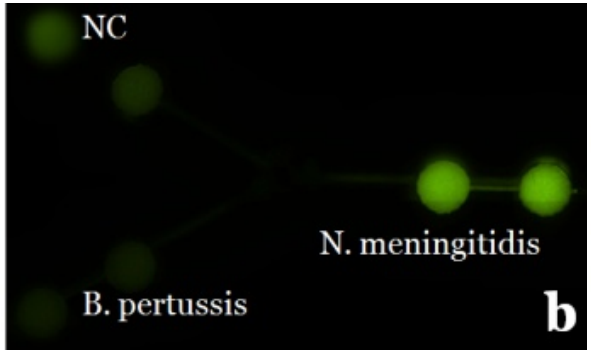


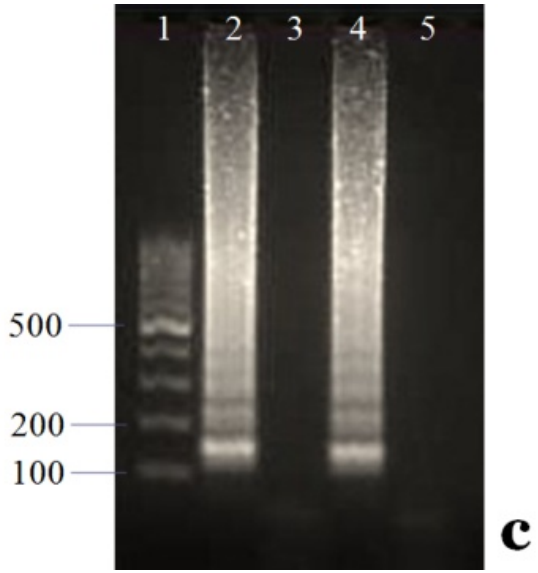

Dou, M, et al, *Anal. Chem*. 2014, 7978

# Specificity Test

- Parasite & bacteria models
  - Causing some similar symptoms

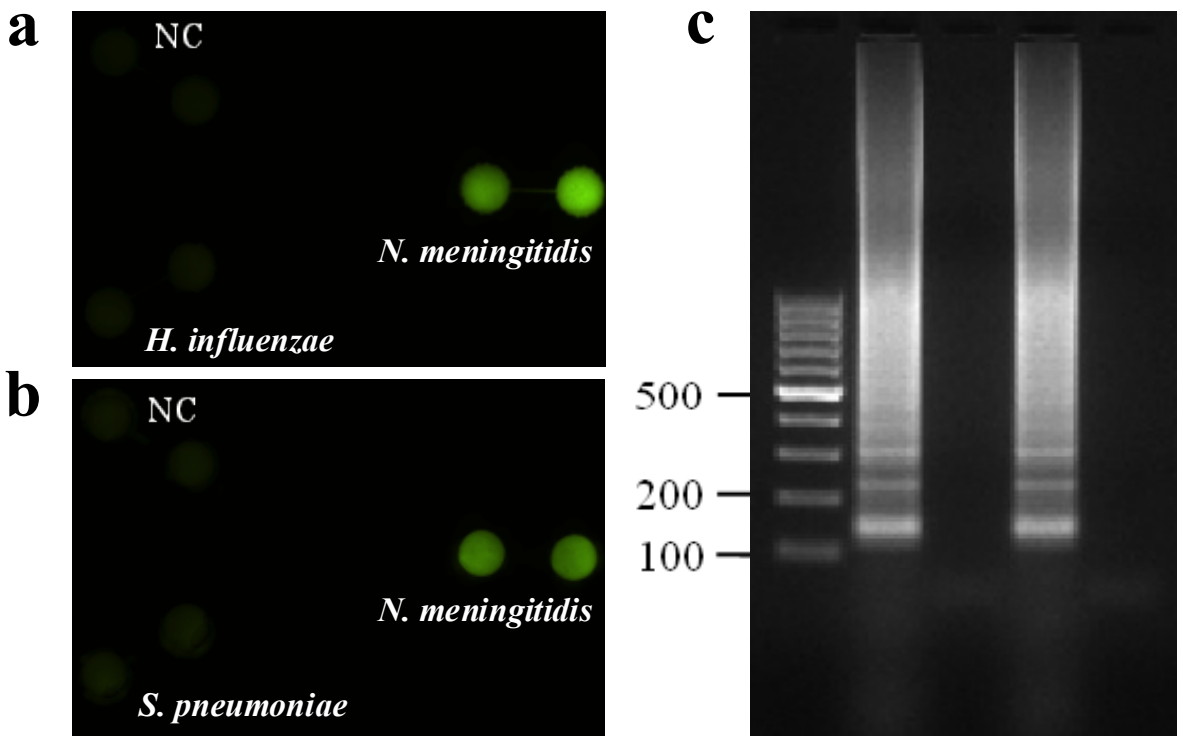


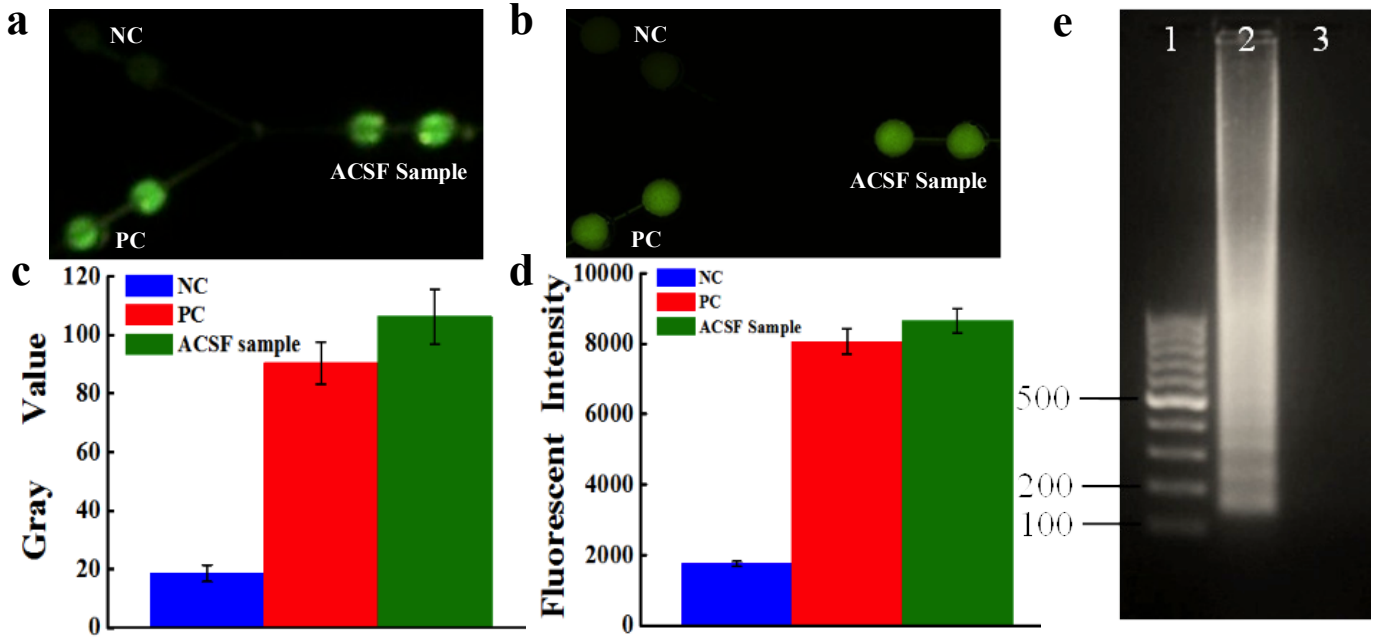
# Specificity Test (continued)

- Compared to 2 meningitis-causing bacteria
- High specificity



# Artificial sample test

- Centrifuge-free DNA extraction
- Artificial cerebrospinal fluid (ACSF)



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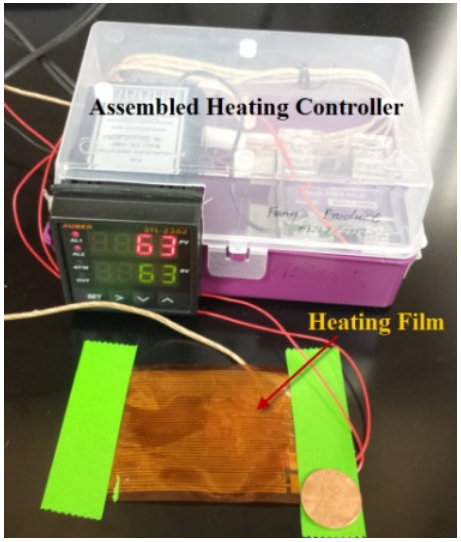
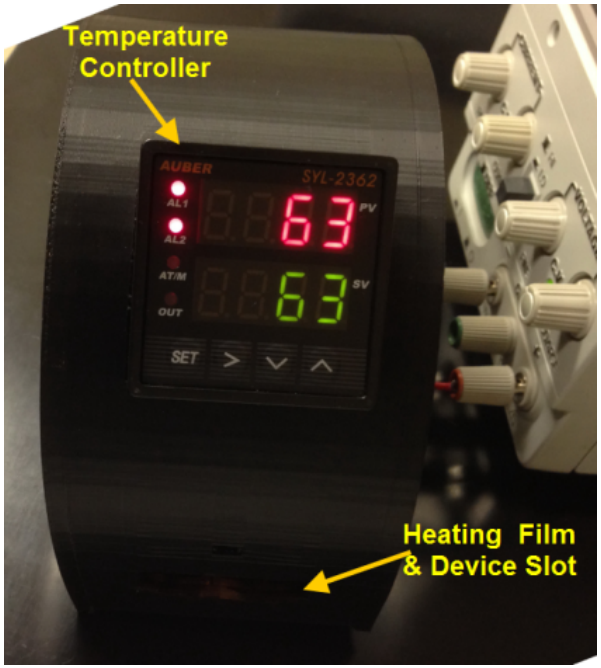
# Portable Heater

- Battery-powered
- 3D printing



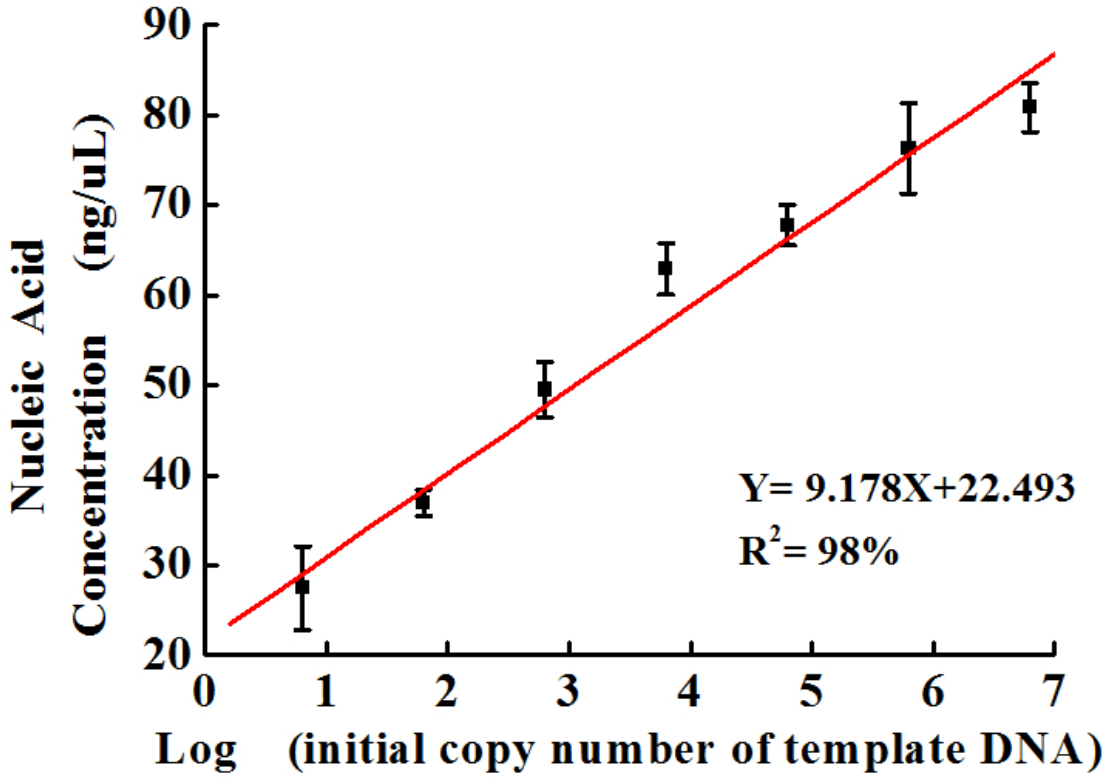

1st generation




2nd generation

3rd generation

# Quantitative Analysis



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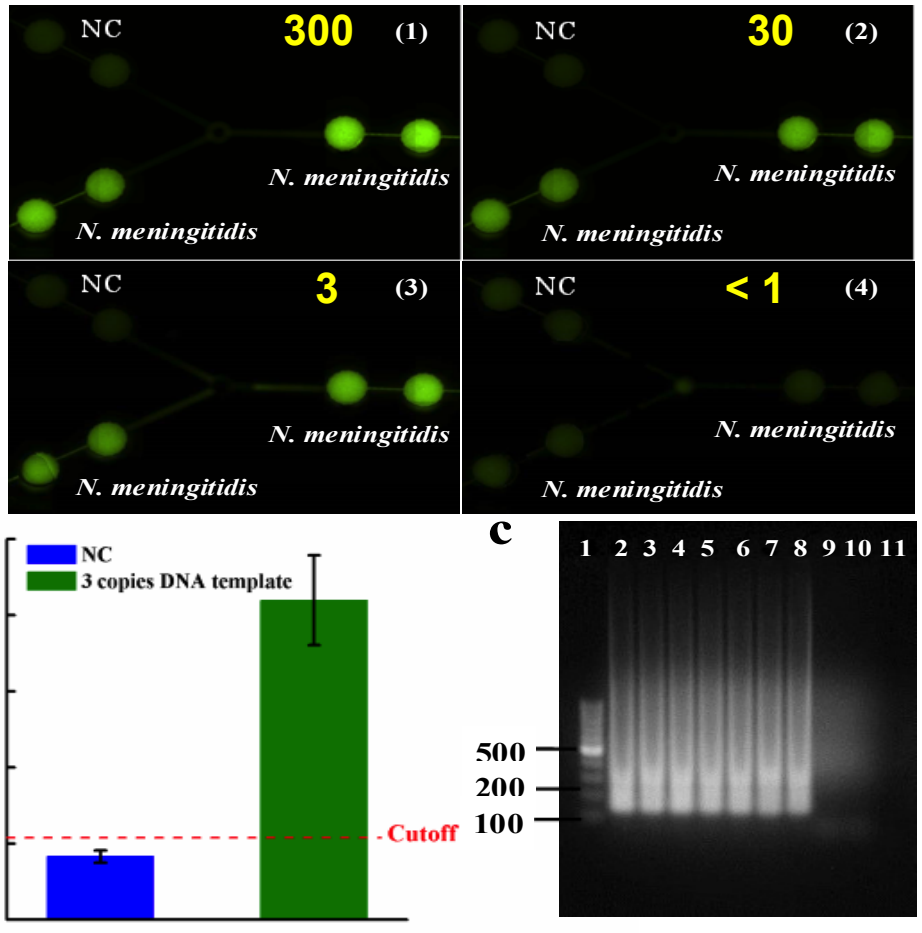
# Limit of Detection (LOD)

- 3 DNA copies per a zone
  - Sensitive than qPCR
- High sensitivity



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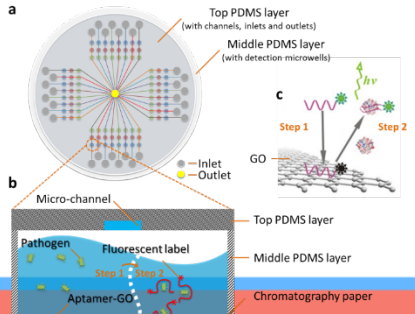
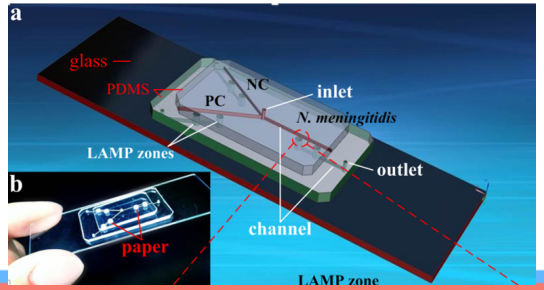
# Conclusions –II.

- Two paper /PDMS **hybrid** microfluidic devices taking advantages of both device substrates have been developed for infectious disease diagnosis.
- Hybrid I.
  - The integrated aptamer-based nanosensors provide a simple one-step method for direct pathogen detection, without cumbersome simple preparation procedures.→ **high simplicity**.
  - Paper facilitates the integration of aptamer-functionalized GO nano-biosensors in a microfluidic system, eliminating complicated surface modification.
  - It is fast. It only takes ~10 min to complete the test using a ready-to-use device.



# Conclusions -II.

- Two paper /PDMS **hybrid** microfluidic devices taking advantages of both device substrate has been developed for infectious disease diagnosis.
- Hybrid II.
  - Although no specialized equipment is used, thanks to the integrated to LAMP on the chip, the method offers very **high detection sensitivity**.
  - The **performance** of the hybrid system is more **stable** than the system without paper inside.
  - The system offers **versatile functions**, suitable for on-site qualitative analysis and confirmatory quantitative analysis.
- Both hybrid systems have great potential in rapid detection of a wide variety of different other pathogens, especially in **low-resource settings**.



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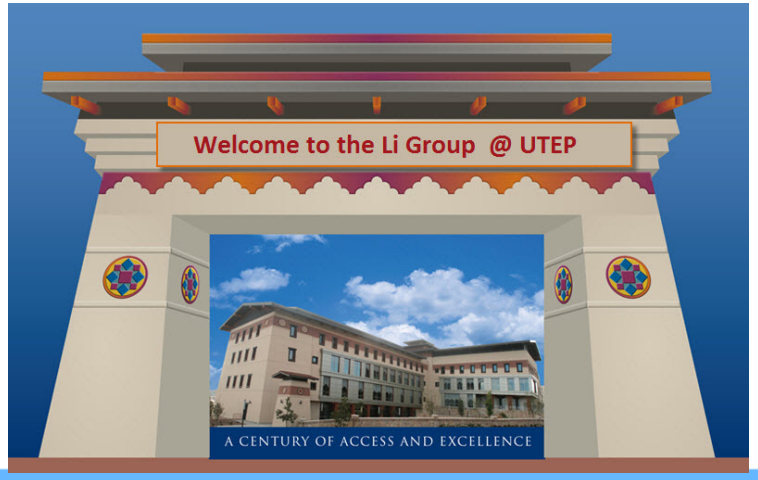
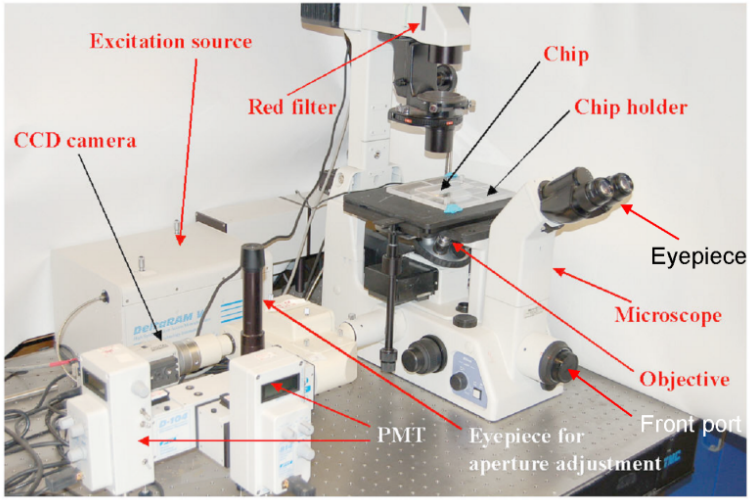


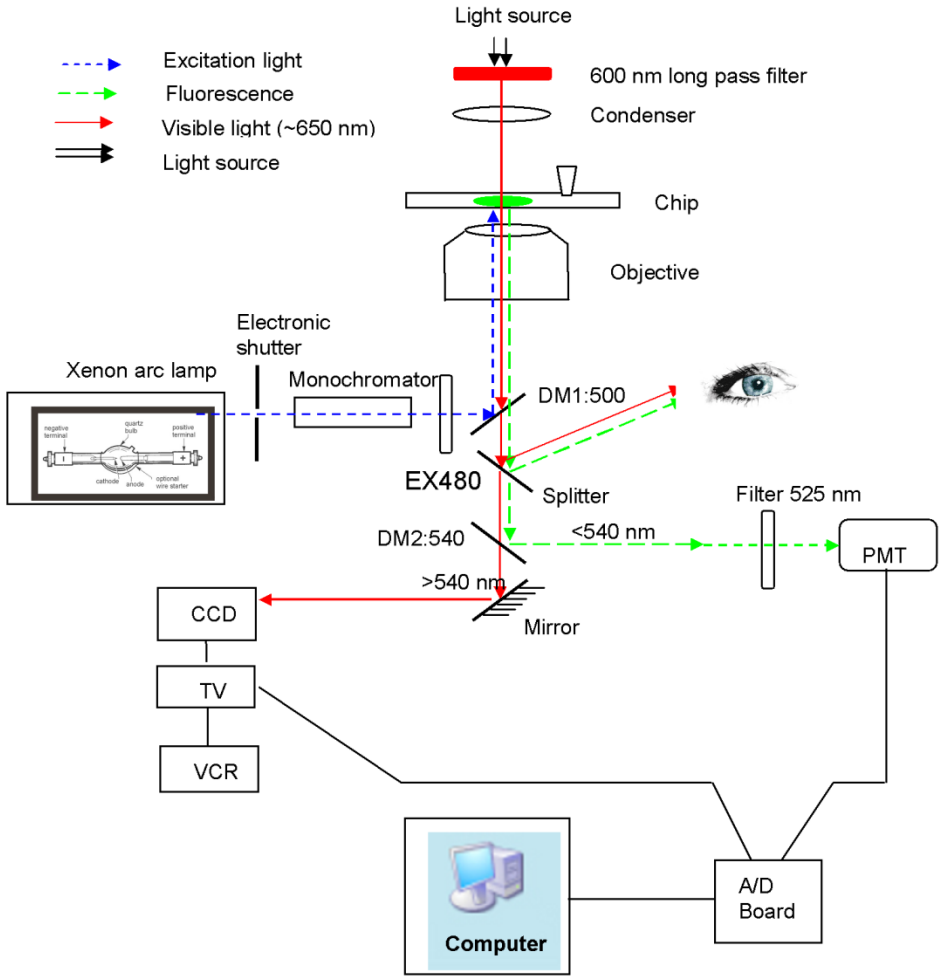
- Elsevier
- http://store.elsevier.com/pro
  locale=en_US&isbn=978085
- Amazon

# Thank you!

# Instrumentation

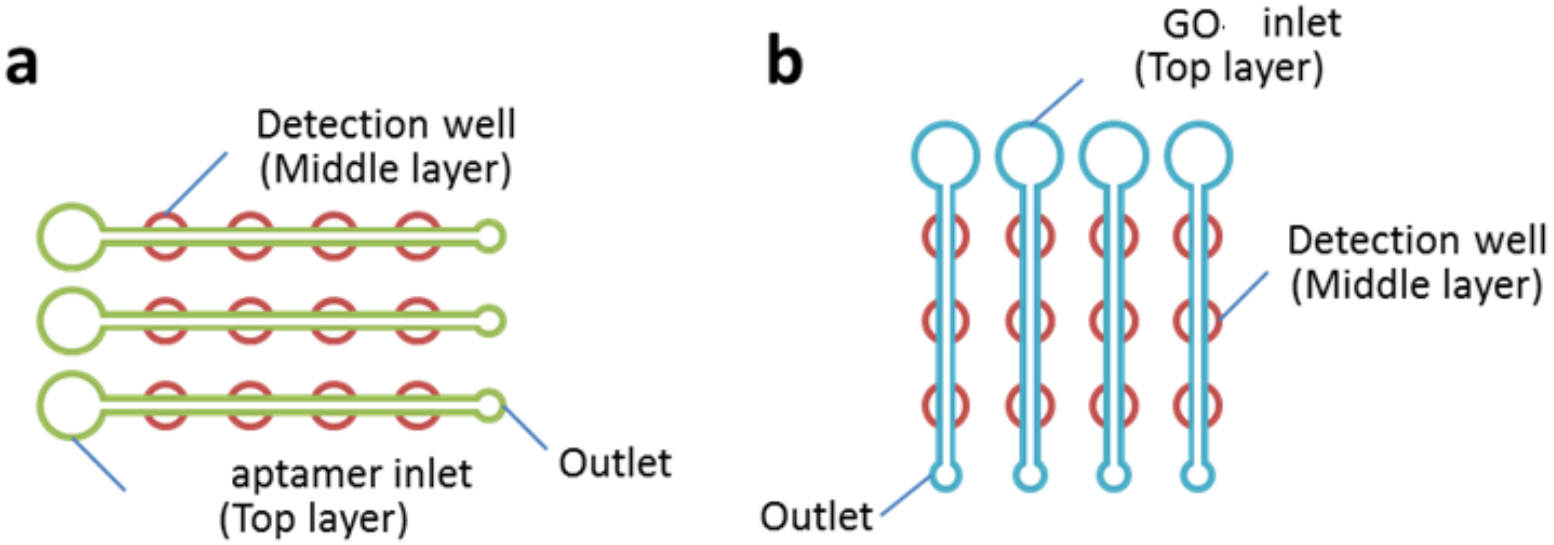
- **Optical Imaging system**
- **Fluorescent measurement system**
- **DM: dichroic mirror**



# Reagent Delivery

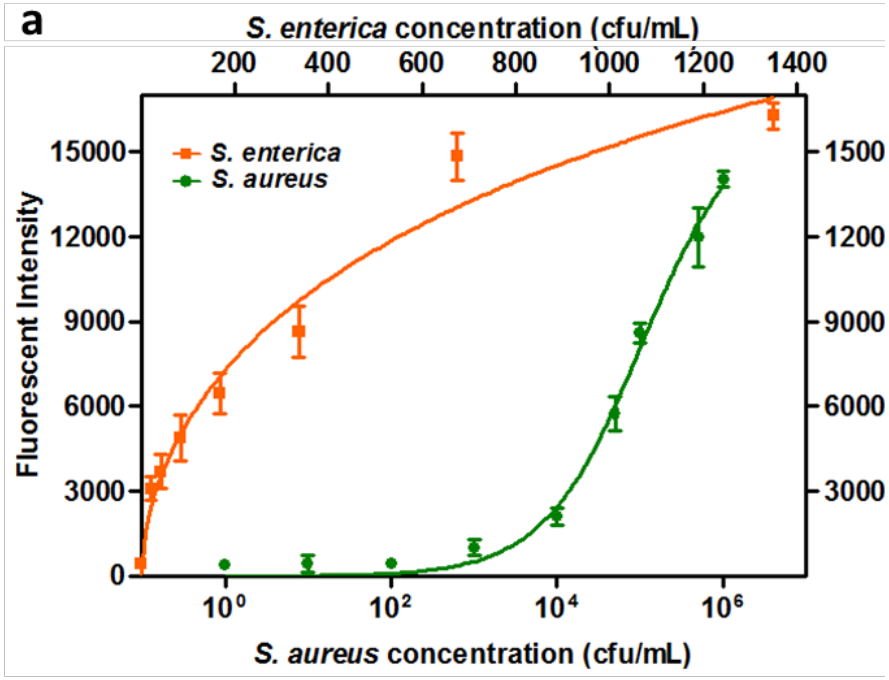
- Avoid repeated micropipetting

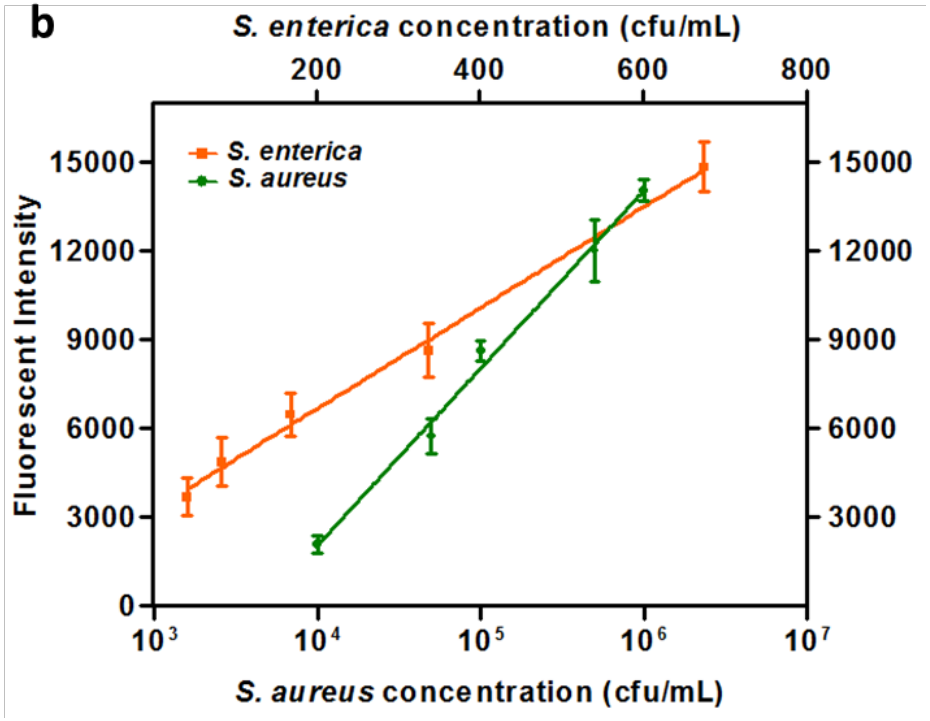





P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*

- LOD, *S. enterica* 61.0 cfu/mL
- LOD, *S. aureus* 800.0 cfu/mL






P. Zuo, X. Li, et al, *Lab Chip, 2013, 13, 3921-3928.*