Introduction to Lab-On-a-Chip system:

實驗室晶片導論

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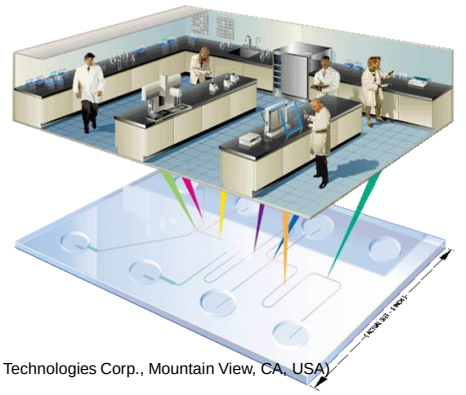
What is Lab on a chip ?

LOAC (or LOC): combining different operations, which are originally performed in laboratories, in a single microdevice. (Berthier & Silberzan)



(From: Caliper Technologies Corp., Mountain View, CA, USA)

Course Organization (I)

實驗室晶片(Lab-on-a-Chip)系統是**將原本在實驗室不同階段之操作流程整合並微小化在一片晶片系統上**。利用這種技術，醫生在幾分鐘的問診過程中可同時快速診斷出病人的疾病，並對症下藥；生化實驗可以減少人因干擾、避免人員直接曝露於有害試劑的危險下工作；另外，實驗室晶片因具有可自動化與平行化操作處理的特色，所以可用於快速篩選或合成新藥與產品，並**增加實驗的可信賴度**；而由於在晶片上僅需極少量的試劑且具表面體積比增大之優點，更可**大幅減少試劑用量、減低操作成本及縮短操作處理時間**。目前已有越來越多的實驗改在實驗室晶片上進行，例如血液分離、電泳分離、聚合酶鏈鎖反應（PCR）、核酸的定序反應分析等等，而拋棄式的塑膠晶片也有漸成設計主流之趨勢。

Course Organization (II)

- 在課程設計上，這是一門讓理、工、電資學院同學伸展觸角到醫、生農等領域的原理與應用課程，也是一門讓醫、生農及其他學院的同學可以實際接觸工程與實作的多樣學習課程。
- Language: Chinese; lecture notes in English
- Lecture Notes on Web: **(**http://bernoulli.iam.ntu.edu.tw/ **)**
- Grading Policy: Class participation (10%);
  1st & 2nd Mid-**term project** presentation (15 + 15%);
  Final oral & written report of **term project** (30%+30%)

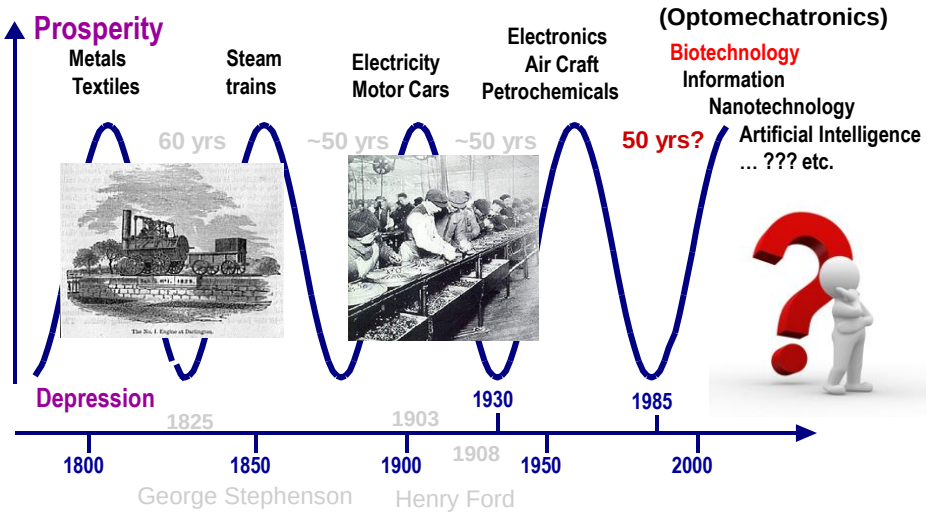
Course Organization (III)

第3、5週將安排在醫學院醫學檢驗暨生物技術學系上課，並參觀臺大基因體中心；第4/10週將安排在臺大奈米機電系統研究中心無塵室實習。

本課程將提供對此一深具未來性之科技有興趣的同學們(大學部及研究所)，一個結合「**理論與實作**」和「**研究與應用**」**四合一**的實際參與機會。

在課程中，除講授實驗室晶片所需的基礎知識、實驗設計與量測方法外，也將讓同學到實驗室動手製做，並邀請不同應用領域的傑出專家，透過其所提出該領域裡的實務問題需求及方向輔導與討論，讓同學們**結合不同專業組成跨領域團隊(每隊1-3人)，以實際動手完成不同的實驗專題**，訓練同學們以**目標為導向**之**團隊合作與邏輯推理能力，同時開啟未來可能之研究方向**。

Trend of the world



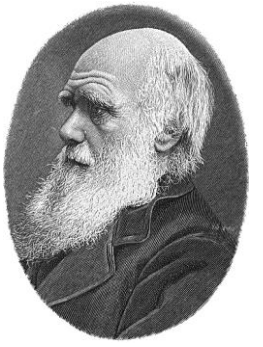
Who can survive in the changing world?

- The one that is the strongest
- The one that is the most intelligent
- The one that is most rich
- The one that is most active
- The one that is most modern
- The one that works very hard
- …

The Simple Answer (I)

It is **not** the ***strongest*** of the species that survives, **nor** the **most intelligent** that survives.

It is the one that is the **most adaptable to change**.

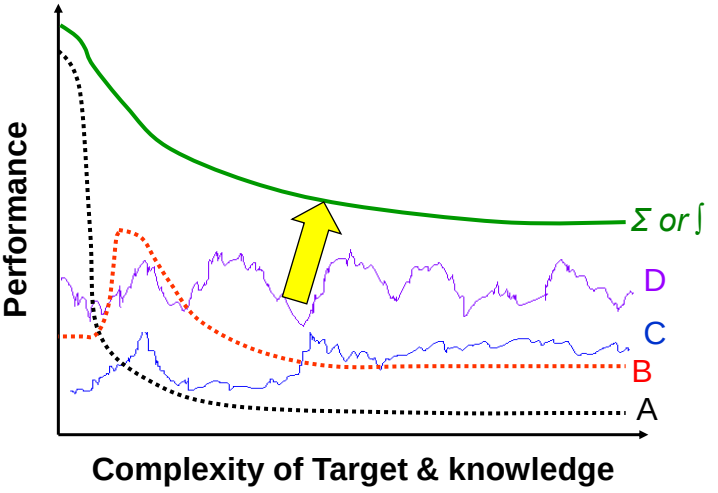


[Charles Darwin
1809~1882*]*

"Adaptable to change"

- What can the "**change**" bring (for you)?
- "**Change**" vs. "**Novelty**"
- What is/are the key(s) of "**adaptable to change**"?
- What is/are the importance of "change" that related to **students/professors**?

Why interdisciplinary?



The Trend of Industry

The trend of industry development depends on the trend of human needs.

- ***Providing Ubiquitous Total solution***
- ***Integration of functionality***
- ***Built in precision/inspection/automation***
- ***Reduce time to certification/ (mass) production/market/profit***

(程一麟)

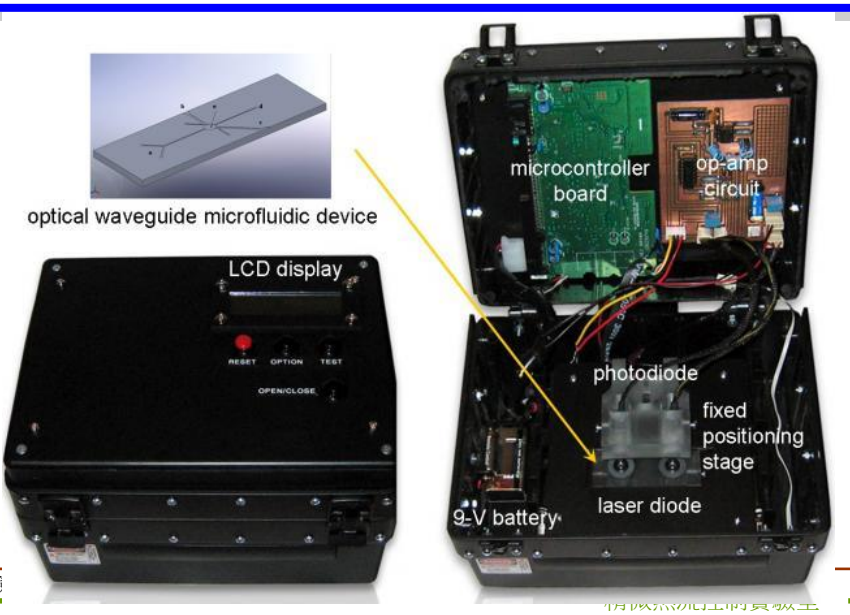
Course Contents (I)

1. 實驗室晶片導論及議題設計介紹
2. 實驗室晶片導論與實驗室晶片議題分配
3. 醫學分子檢驗新技術 I
4. 微製程技術簡介 & 實作(I) : MEMS實作篇(A)
5. 醫學分子檢驗新技術 II (& Lab course 傳統實用篇)
6. 微流體混合/反應暨生醫化材應用
7. 用於生物樣本前處理之微流道系統
8. 光流體系統簡介
9. 第一次期中報告與實驗室分組實作

Course Contents (II)

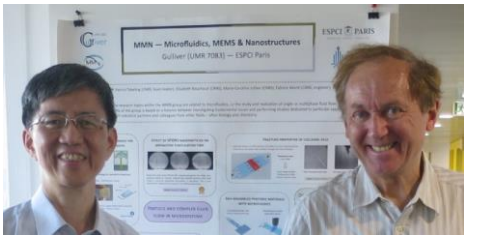
10. 實驗室晶片設計與實作(III): MEMS實作篇(B)
11. 肝臟與肝臟晶片技術簡介
12. 肝臟晶片實際應用與未來展望
13. 第二次期中討論與報告
14. 塑膠基材之微流體感測器
15. 紙張基材之微流體感測器
16. 神經訊號傳遞
17. 液珠輸送與檢測晶片
18. 期末報告

Lab-on-a-chip system



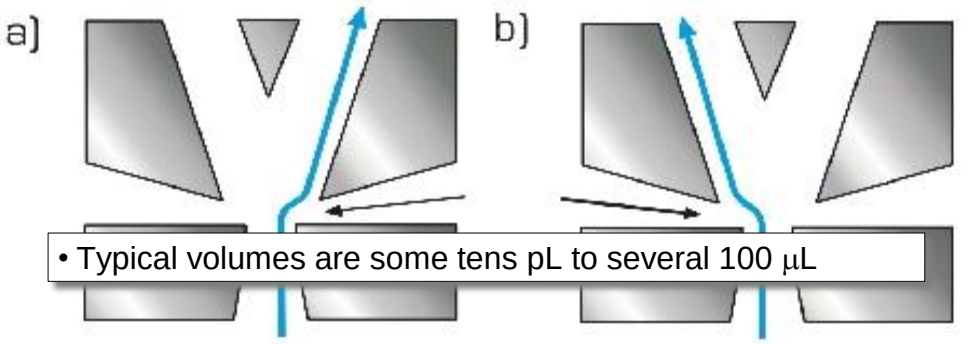
What are *LOAC* & *μ-fluidics*?

- ***There are different names used in the literature: μ-fluidic, MEMS-fluidics, μ-TAS, BioMEMS, biochip, LOAC, nanofluidics, nanoflows… etc.***
- ***μ-fluidic*** is the study of flows, which are circulating in artificial ***μ-***systems. (Prof. **Patrick Tabeling**)
- ***μ-TAS***: ***M***icro **T**otal **A**nalysis **S**ystems



What is Microfluidic technology ?

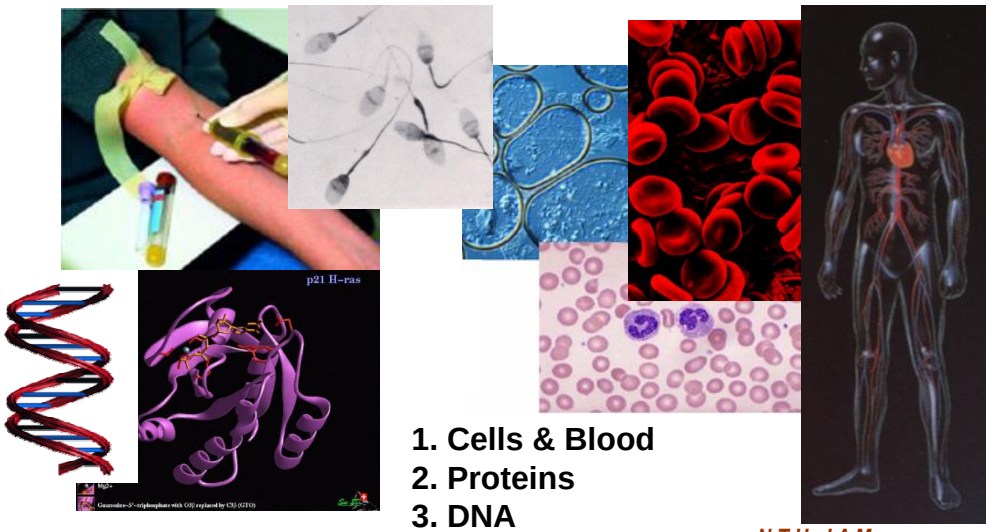
◆ **Microfluidic = "Micro" + "Fluidic"** (fluids manipulation)



• Typical volumes are some tens pL to several 100 μL

◆ **Different names for μ-fluidic: LOAC, biochip, MEMS-fluidics, nanofluidics, μ-TAS (Total Analysis Systems) etc.**

Biological Fluids



1. Cells & Blood
2. Proteins
3. DNA

Introduction to Surface Tension

Surface tension is the energy required to increase the interface of two immiscible fluids by an unit area.

Surface tension is the force applied along the interface of two immiscible fluids per unit length.

http://itsforyourlife.com/

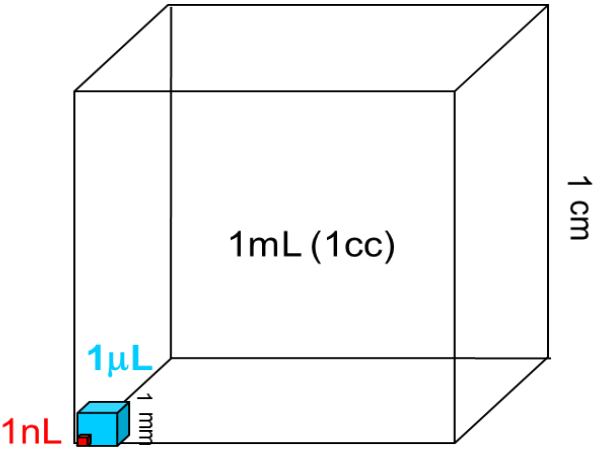
http://www.liv.ac.uk/

http://fphoto.photoshelter.com/

http://www.natoco.co.jp/

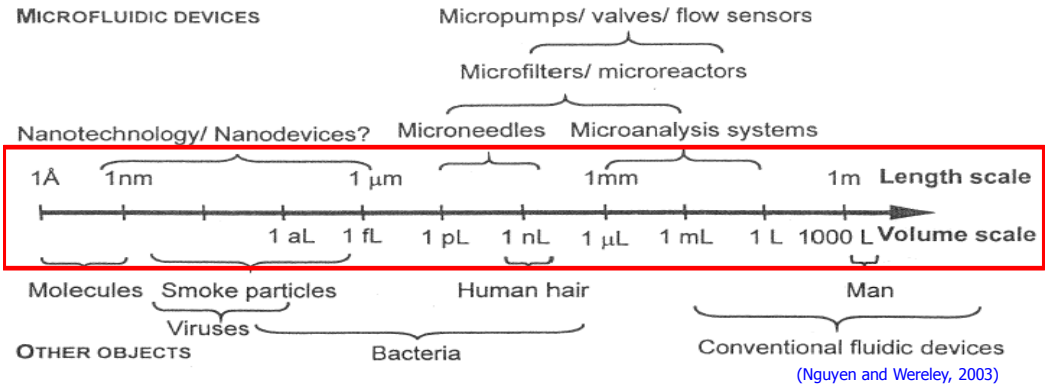
How large is one drop size by a typical eyedropper?

1. 5 mL
2. 0.5 mL
3. 0.05 mL
4. 0.005 mL
5. 0.0005 mL



Why a microfluidic platform?

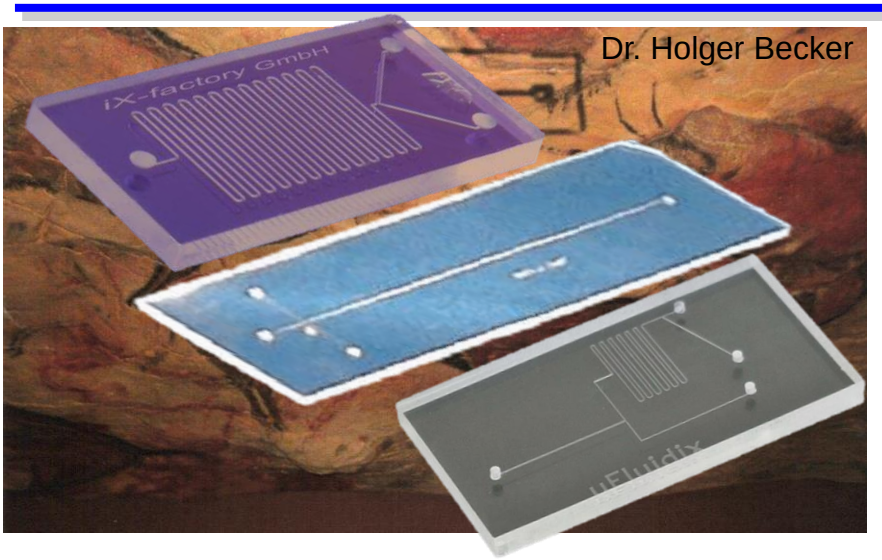
- Length scale & volume



(Nguyen and Wereley, 2003)

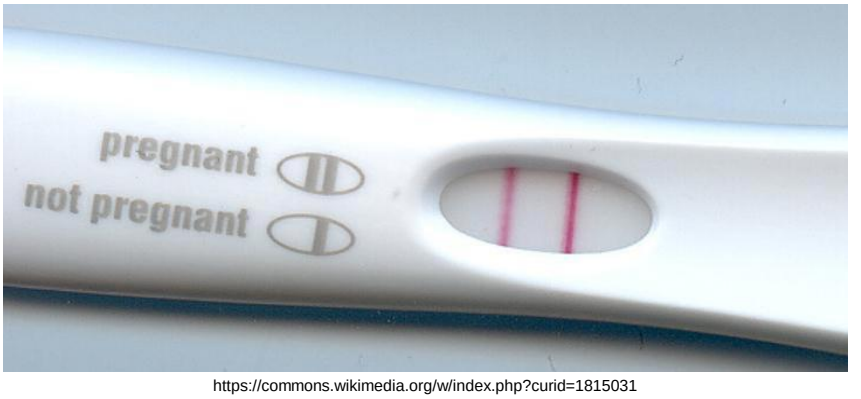
How to precisely metering a tiny drop?

Microfluidic has been around for a long time?



The pregnancy strip is a modern/traditional test ?

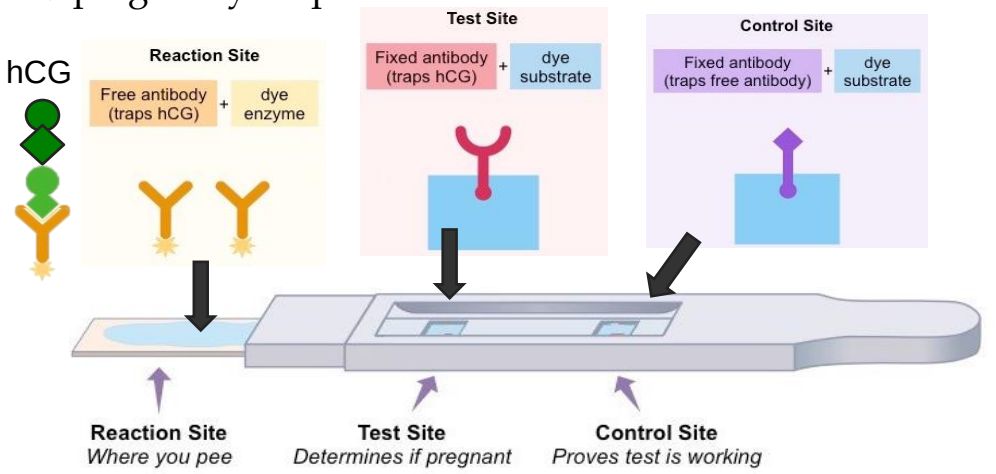
- The product became available in Canada **in 1971**
- This is a test of **hCG** (human chorionic gonadotropin)



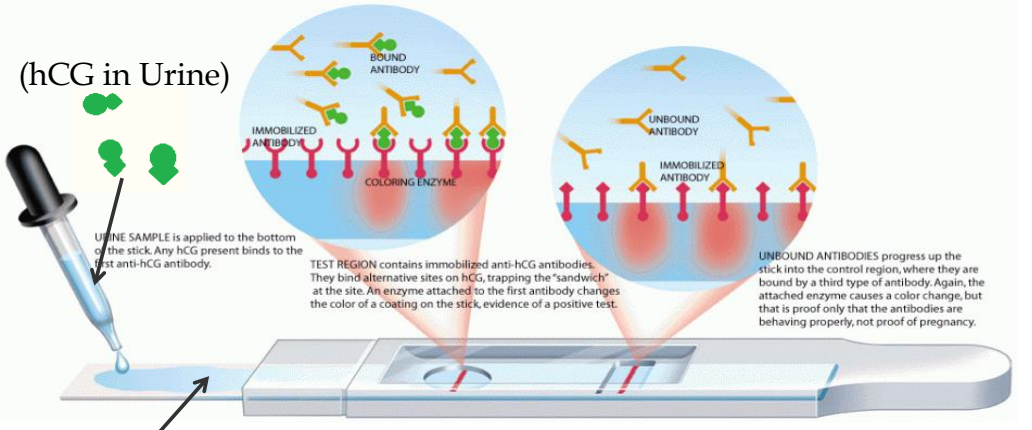
https://commons.wikimedia.org/w/index.php?curid=1815031

The sensor design of a pregnancy strip

◆ pregnancy strip is a nanomaterial-based sensor



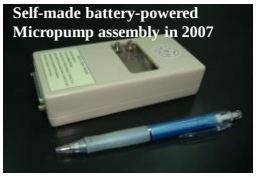
How a pregnancy strip works?



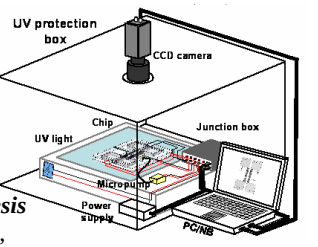
In microfluidic terminology:
lateral flow transportation

https://pregnancy.biz/pregnancy-tests/

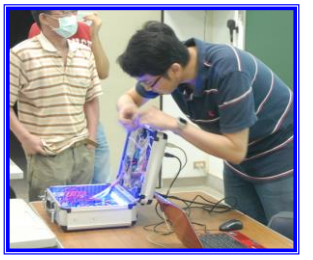
Examples of Past Term Projects



published in
Sensors and Actuators –B: Chemical, 2016, Vol. 222 pp. 721–727



published in
Electrophoresis 2011, Vol. 32, p.423-430.



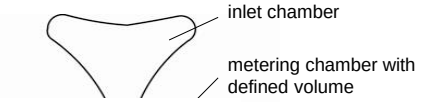
Gravity-actuated Microfluidic Chip for Point-of-Care Urinary Creatinine Detection

Sensors and Actuators –B: Chemical, 222 pp. 721–727, 2016

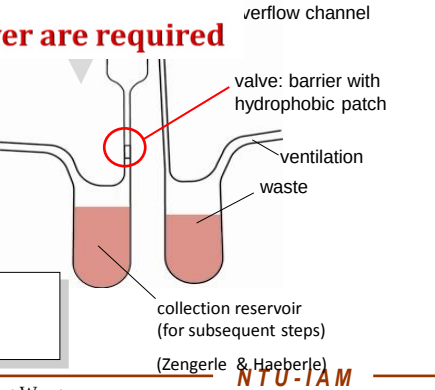
Centrifugal μ-Fluidics on Disk (I)

1. **liquid metering**
2. liquid switch
3. separation
4. mixing
5. detection

$$\boldsymbol{f}_{\nu} = -\varrho\boldsymbol{\omega} \times (\boldsymbol{\omega} \times \boldsymbol{r})$$



- **Patent issue**
- **Stable motor & power are required**



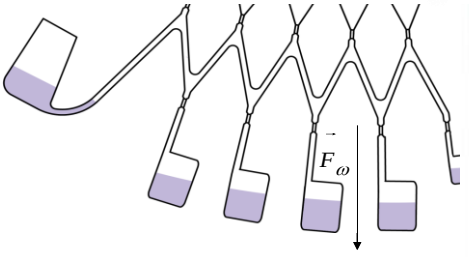
- Control by rotating speed
- Disposable & Low cost

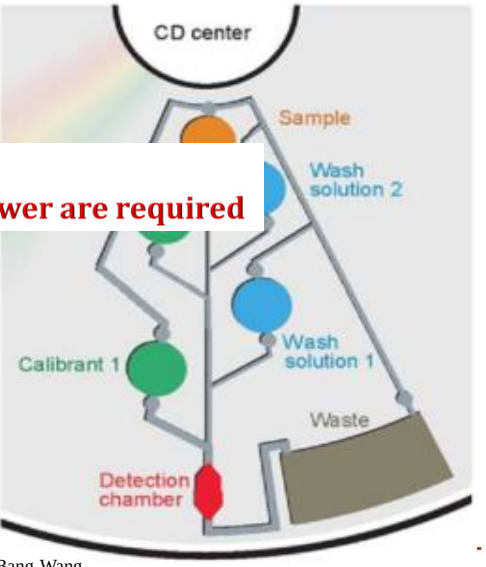
(Zengerle & Haeberle)

Centrifugal μ-Fluidics on Disk (II)

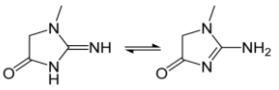
Splitting/Aliquoting for multi-channels

- **Patent issue**
- **Stable motor & power are required**



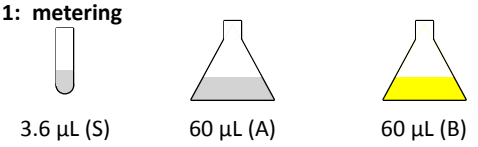


Target: Urinary Creatinine Assay (collaborated with NTU-Hospital)



Creatinine is produced at a fairly constant rate by muscle, and its concentration in blood or urine is a standard to evaluate the renal function.

Step 1: metering



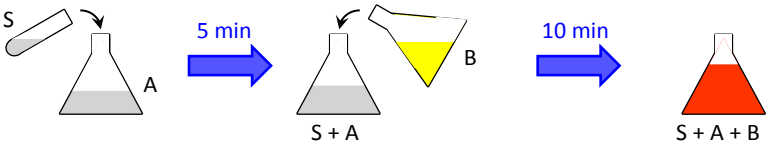
Sample preparation & precise metering:

a. 3.6 µL test sample (S)
b. 60 µL reagent A (A)
c. 60 µL reagent B (B)

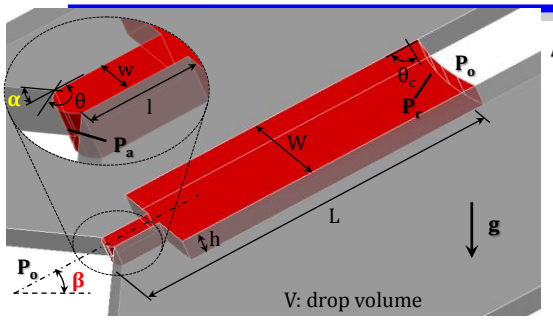
Step 2:
S + A mixing

Step 3:
(S + A) + B mixing

Step 4:
Mixture becomes reddish orange & then detection



Capillary-Gravitational Valve



$$\Delta P_a = P_o - P_a = 2\sigma\left[\frac{\cos(\theta_c+\alpha)}{w}+\frac{\cos\theta_c}{h}\right]$$

$$\Delta P_r = P_r - P_o = -2\sigma\cos\theta_c\left(\frac{1}{w}+\frac{1}{h}\right)$$

$$\Delta P_g = \Delta P_a + \Delta P_r = -\rho g L \sin\beta$$

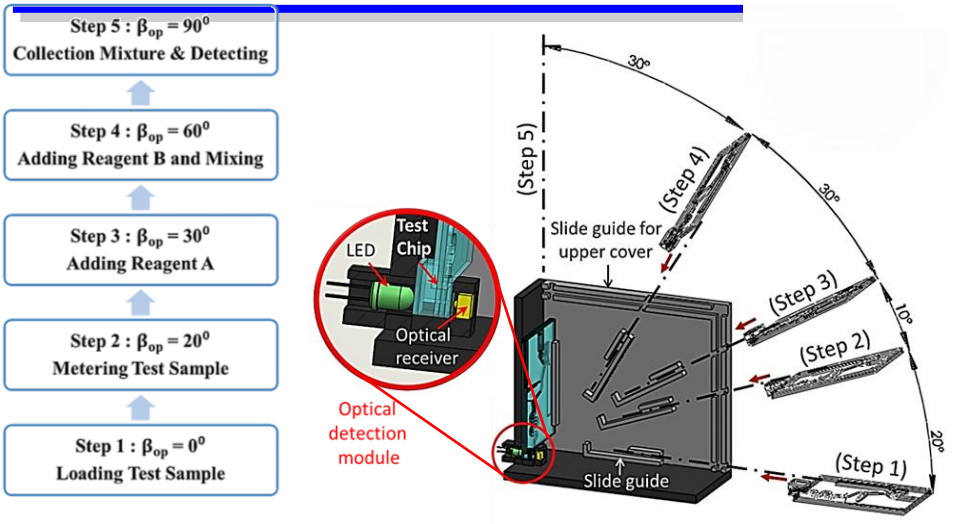
where $$L = \frac{\frac{V}{h}+(W-w)l}{W}$$

(Sensors and Actuators –B: Chemical, 222, pp. 721–727, 2016)

$$\beta_{op} = \sin^{-1}\left\{\frac{2\sigma W}{\rho g\left[\frac{V}{h}+(W-w)l\right]}\left[\frac{\cos\theta_c}{W}-\frac{\cos(\theta_c+\alpha)}{w}\right]\right\}$$

With an appropriate set of geometric design (**α**, w, W, h, and l), sequential control can be realized by simply changing β_{op}.

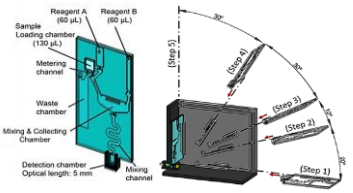
Lab-on-a-chip for point-of-care of Urine test



(TW Patent I446958)

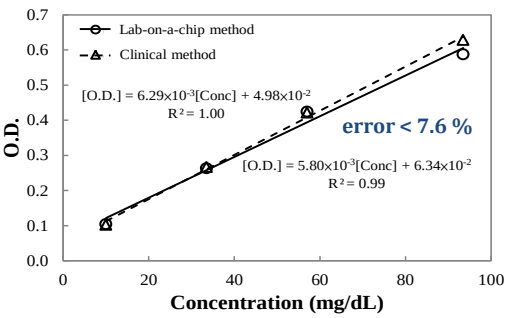
Urine Chip vs. Clinical Method

Lab-on-a-chip method:



Clinical method:

(TBA-200FR, Toshiba)



The new design is suitable for POCT (<10%) in remote areas even without electricity.

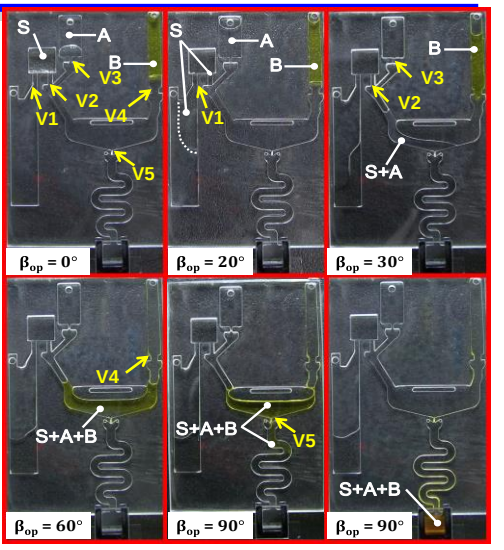
Sensors and Actuators –B: Chemical, 222 pp. 721–727, 2016

Functional Test of Urine-Chip

*The chip is placed on the rotating stage in the horizontal plane.

*Load reagent A , reagent B , and urine sample.

channel

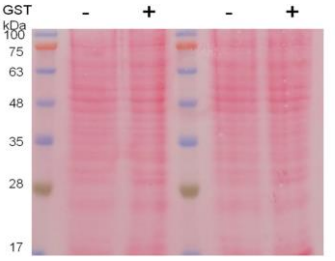


0˚: load samples
20˚: open **V1** (S precise metering)
30˚: open **V2 & V3** (S + A)
60˚: open **V4** (S + A + B)
90˚: open **V5** (detecting)

A Novel DNA

Selection and Direct Extraction (SDE) Process and its Application in DNA recombination

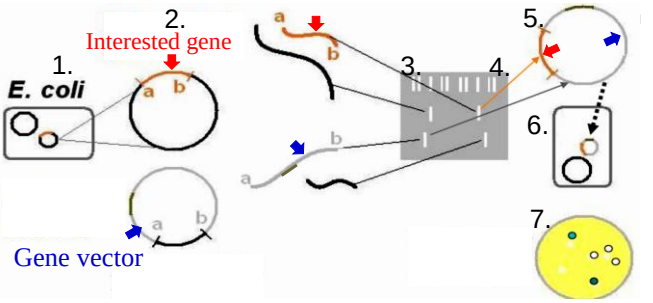
(Electrophoresis, 2011, 32, pp. 423–430)



DNA Recombination

Lab process

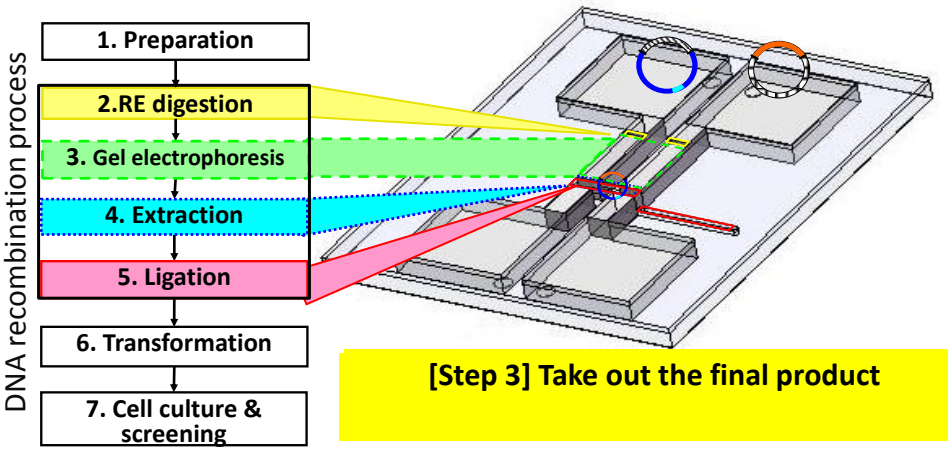
1. DNA preparation (~1 hr)
2. RE digestion (1~2 hr)
3. Gel electrophoresis (~1 hr)
4. DNA extraction (~1 hr)
5. DNA ligation (1~20 hr)
6. Transformation (1~2day)
7. Cell culture & selection (~4 hr)



◆ **Demerits：**

1. Low production rate
2. Time-consuming
3. Hard manipulation of large DNA
4. Exposure to dangers (EtBr, UV light)

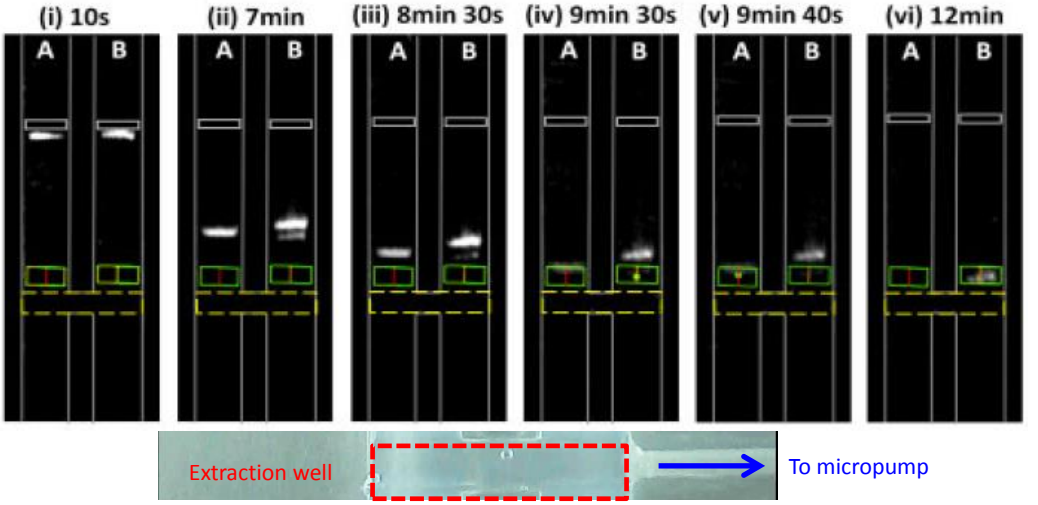
Chip design



[Step 3] Take out the final product

Multi-steps of DNA recombination into μ-fluidic chip

Demonstration

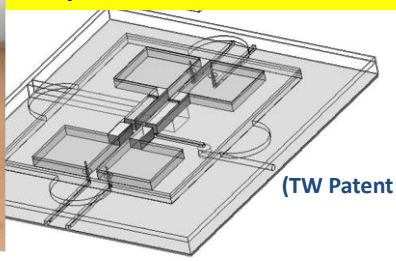


System overview

Microfluidic chip + Automatic control box

[Characteristics]

- **Continuous electrophoresis & extraction**
- **Microfluidic mixing & control**
- **Precision thermal control of reaction**
- **Real-time image processing**
- **Fully automatic control**
- **Friendly user interface**



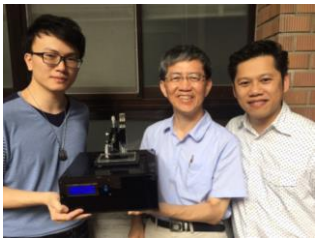
(TW Patent I358539)

Comparisons

	Traditional technique	Present LOC
Operation time	~1 day	< 1 hr
Amounts of DNA	3-5 mg	< 1 mg
Manual checkpoints	> 10	0
Gel cut	YES	NO
Gel extraction pack	YES	NO
Exposure to UV	~1 min/sample	NO
Multiple samples in parallel	Increase manpower	Easy by fully-automatic control
Manipulation of large DNA	Difficult	Feasible (> 10kb DNA vector)

Novel Biomedical Detection Easy & Fast Western Blotting by Thin-Film Direct Coating

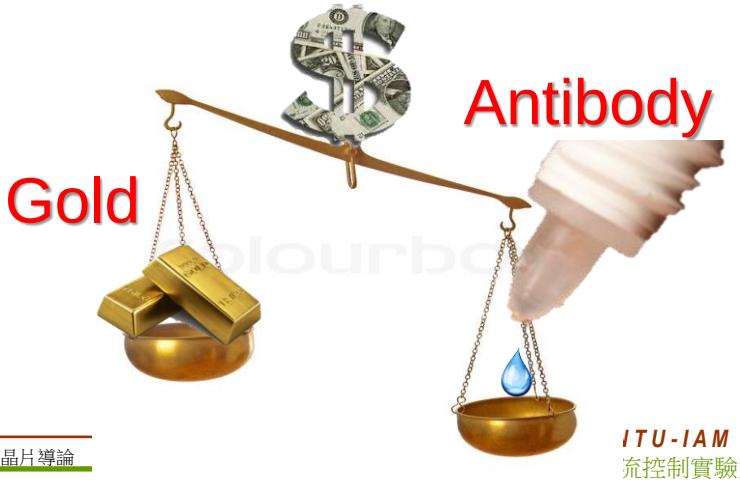
Analytical Chemistry, **86**, pp. 5164-5170, 2014
Analytical Chemistry, **88**, pp. 6349–6356, 2016.



The world smallest & lightest slot-die precision coating system

Characteristics of biomedical fluids

- generally **expensive** or even **limited**
- **Precision** is strictly needed



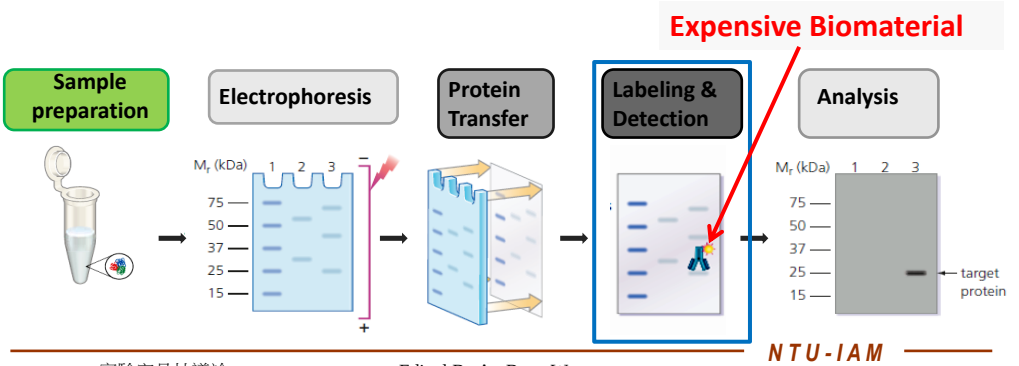
Antibody-based Immuno-detection methods

Name	Users
Western blotting, WB	**Research Institute / Lab**
Enzyme-linked immunosorbent assay, ELISA	**Research Institute / Lab / Hospital**
Immunohistochemistry, IHC	**Research Institute / Lab / Hospital**
Immunocytochemistry, ICC	**Research Institute / Lab / Hospital**
Turbidimetry	**Hospital**
Others	**Research Institute / Lab / Hospital**

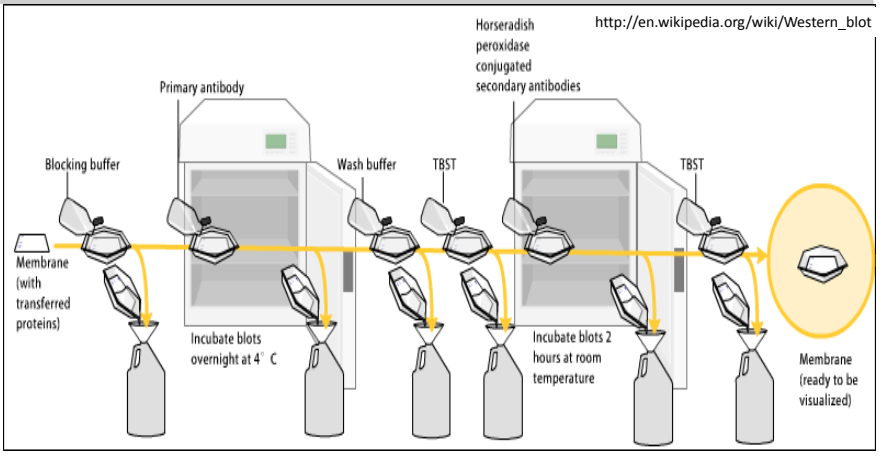
μ–fluidic -based Detection

Example: Western Blotting

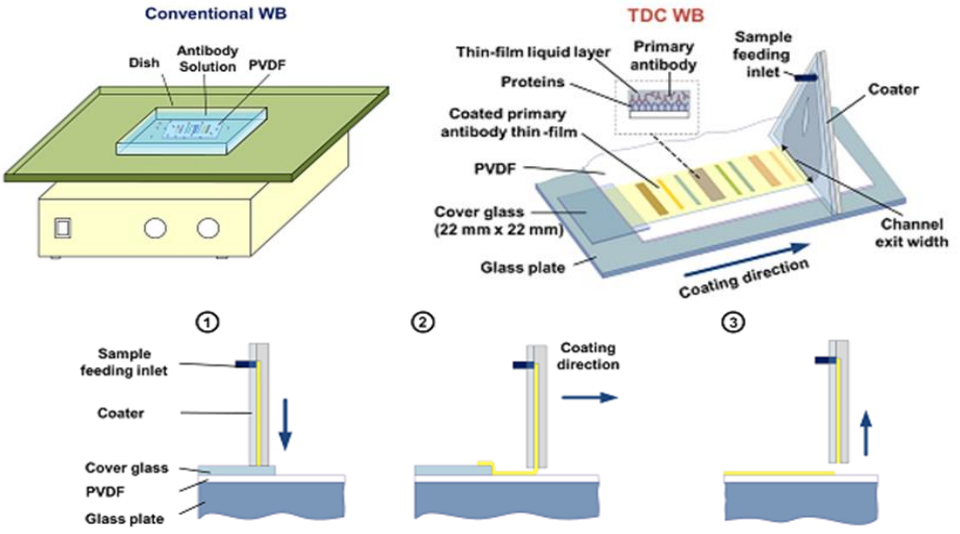
- Western blotting, also known as immunoblotting or protein blotting, is a core technique in cell and molecular biology
  - Detecting the presence of a specific protein in a complex mixture extracted from cells
  - Being widely used for the test of protein expression level



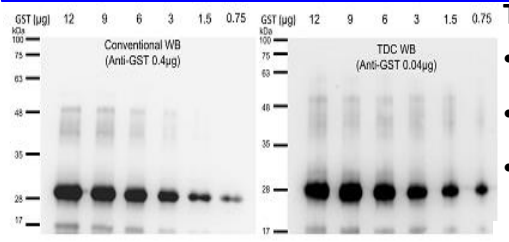
There are many tedious processes…



Thin-Film Direct Coating Western Blotting (TDC WB)

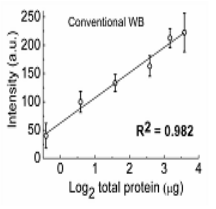


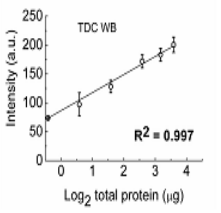
Performance Test of TDC WB

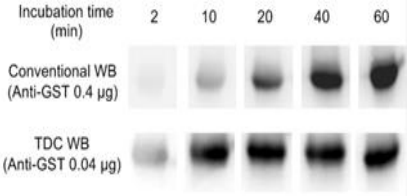


TDC WB vs. Conv. WB:

- **Better SNR**
- **less material needed**
- **Shorter time**



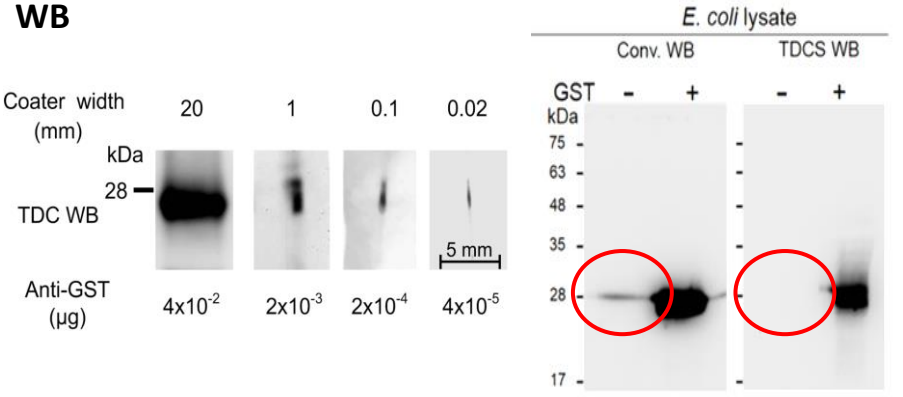




(Analytical Chemistry, 2014)

Performance Test of TDC WB

- **Reducing coating width of TDC WB**
- **Reducing false signal**



Performance Test of TDCS WB

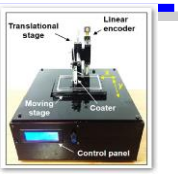
- **By adding Suction after TDC coating (TDCS): material consumption: 1/100 ~ 1/10,000 operation time: 1/36**
- **Automatic platform**

	Convention WB	TDCS WB
Ab (μg)	2	0.02~0.0002
time(min)	180	≤ 5
Multi-Ab	No	Yes
SNR	good	excellent

(Analytical Chemistry, 2016)

Performance comparison



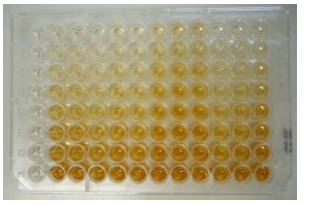



	Traditional Western	Merck Millipore	Thermo Fisher Scientific	AB Wang's Group
Antibody consumption	100%	100%	20%	≤ 1%
Operation time (hr)	3 hr ~ overnight	1/2 hr	2.5 hr ~ overnight	1/12 ~ 1/5 hr
Automatic sequential operation	No	No	Yes	Yes

目前市場上無可同時省料、省時與省人力之產品!!!

Term projects in 2018/9:

1. Development of Quantum Dot microreactor & detection platform
   工業用量子點材料微反應器與檢測平台開發
2. Smart Contact lens for monitoring Chronic diseases
   智慧型醫用隱形眼鏡系統開發
3. High performance Western Blotting Integration System
   高效西方墨點法檢測平台開發
4. New enzyme-linked immunosorbent assay (ELISA) system
   全新長效高精度植入式藥物施打系統
5. qPCR



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***8.* Journal, conference papers, seminars and information from Webs.**