

行政院國家科學委員會專題研究計畫 期末報告

設計, 組裝及自動化控制微流體晶片平台在生化分析之應用

計畫類別：個別型
計畫編號：NSC 101-2113-M-040-002-
執行期間：101年08月01日至102年07月31日
執行單位：中山醫學大學應用化學系(所)

計畫主持人：萬金鳳

計畫參與人員：大專生-兼任助理人員：羅章耘
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大專生-兼任助理人員：徐偉強
大專生-兼任助理人員：劉東諤
大專生-兼任助理人員：王靖鋒

公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢

中華民國 102 年 10 月 29 日

中文摘要：整合生物分析或化學反應於微流體晶片平台具有許多的優點，例如樣品的自動化操作，實驗室分析系統的微小化及樣品的微量化，以及靈敏的偵測方式均可整合在單一的微小化的元件中(實驗室晶片)以完成實驗室微量的操作。另外，液體的行為在微尺吋下對於分子的擴散及分子之間的作用可獲得良好的控制。除此之外，多種樣品試劑的高通量分析實驗在一個微小化的元件中完成，可降低使用化學藥品的費用以及減少化學廢液的產生量。本研究主要的目標共分為三大部分：(I)第一為紫外光光路系統，此平台包含有光源、光路的設計以及構築於微機電系統之微鏡面陣列，用以調控光束的準直度及光陣列的準確位置。(II)設計，組裝以及自動化控制微流體晶片平台的建構，以應用於平行化分析多種試劑及數個步驟的生物或化學的反應流程程序。(III)整合以上兩個次系統之光學光路平台及微流體晶片平台之組件及功能在同一個系統中。此新開發之光學光路平台與微流體晶片平台可用於蛋白質的檢測系統，鍵結酵素於陣列式光阻表面，用以定量並偵測檢驗樣品溶液中酵素動力學。

中文關鍵詞：微流體晶片平台(microfluidic chip platform)，微機電系統(Micro Electro Mechanical Systems, MEMS)，微鏡面陣列(micromirror arrays)

英文摘要：The integration of biological assays and chemical reactions into microfluidic chip platforms has numerous advantages including automated sample processing, miniaturized analytical system, and sensitive detection onto a single microdevice. Moreover, the unique behavior of liquids on micro-scale allows better control of molecular diffusion and interactions. In addition to high through-put analysis, performing experiments on a micro-device will also reduce the cost of purchasing chemicals as well as the amounts of chemical wastes. This research includes the following three portions: (I) the development of UV optical path platform consisting of the core components of MEMS (Micro Electro Mechanical Systems), such as micromirror arrays and the light source, as well as its control for beam steering and accurate positioning of optical microarray; (II) the design, fabrication and automatic control of microfluidic chip platform, which could be used for parallel assays with multiple

reagents to achieve multistep biological and/or chemical processes; (III) the integration of optical and microfluidic chip platforms as well as the automation of the entire system for performing high through-put biological and/or chemical processes on a microfluidic chip. This newly developed optical and microfluidic platform was utilized as a protein detection system by immobilizing glycohydrolase on photoresistant surface to quantify glycohydrolase kinetic in sample solution.

英文關鍵詞： 微流體晶片平台(microfluidic chip platform), 微機電系統 (Micro Electro Mechanical Systems, MEMS), 微鏡面陣列 (micromirror arrays)

行政院國家科學委員會補助

期中進度報告

專題研究計畫

期末報告

設計組裝及自動化控制微流體晶片平台在生化分析之應用

Design, fabrication and automatic control of microfluidic chip
platform for bioassay

計畫類別： 個別型計畫 整合型計畫

計畫編號：NSC 101-2113-M-040-002-

執行期間：101 年 08 月 01 日至 102 年 07 月 31 日

執行機構及系所：中山醫學大學應用化學系所

計畫主持人：萬金鳳 助理教授(中山醫學大學應用化學系所)

計畫參與人員：萬金鳳, 羅章耘, 劉柏慶, 徐偉強, 古忠文, 彭崇文,
鄭力遠, 洪士勳, 王靖鋒, 李修慎, 劉東諤

本計畫除繳交成果報告外, 另含下列出國報告, 共 0 份:

移地研究心得報告

出席國際學術會議心得報告

國際合作研究計畫國外研究報告

處理方式：除列管計畫及下列情形者外, 得立即公開查詢

涉及專利或其他智慧財產權, 一年 二年後可公開查詢

中 華 民 國 102 年 10 月 23 日

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以100字為限）

實驗失敗

因故實驗中斷

其他原因

說明：1. 已達成目標。

2. 研究之設備系統在中山醫大重新建構,重新架設大約一半,目前僅利用現有設備進行實驗.

2. 研究成果在學術期刊發表或申請專利等情形：

論文： 已發表 未發表之文稿 撰寫中 無

專利： 已獲得 申請中 無

技轉： 已技轉 洽談中 無

其他：（以100字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）

本研究結合微機電系統工程、化學合成技術及生物化學以及生物科技之基本知識，對於未來科技研究工作之發展甚具潛能，並兼具論文發表之學術性。生物晶片應用在疾病檢測、醫藥的篩選等對於未來之生物醫學將有莫大之幫助。

本計畫在學術研究方面之具體發展為：

- 本計畫所研發之晶片系統可應用於微量之生物分子之定量分析。
- 本計畫所研發之晶片系統可應用於微量之酵素反應動力學的觀測。
- 本計畫所研發之微流體晶片系統可應用於藥物之開發。
- 本計畫所研發之微流體晶片系統可結合本校醫學大學之特色，應用於生醫檢測分析。

可發展之相關技術包含有半導體製程技術之應用、微系統加工技術研發、微結構之分析設計、醣類合成化學在微系統之應用、蛋白質體與微系統之整合以及生物檢測系統技術之發展。此微流體晶片為生醫之重要檢測工具，可申請專利，發表期刊，並投入產業界，同時應用於日常生活中。

國科會補助計畫衍生研發成果推廣資料表

日期：__年__月__日

國科會補助計畫	計畫名稱: 設計組裝及自動化控制微流體晶片平台在生化分析之應用 計畫主持人: 萬金鳳 助理教授 計畫編號: NSC 101-2113-M-040-002- 領域: 自然處, 感測器與微型分析系統		
研發成果名稱	(中文) 說明於後面結案報告中.		
	(英文)		
成果歸屬機構		發明人 (創作人)	
技術說明	(中文) 說明於後面結案報告中. (200-500字)		
	(英文)		
產業別	相關技術包含有半導體製程技術之應用、微系統加工技術研發、微結構之分析設計、酵素反應動力學在微系統之應用、蛋白質體與微系統之整合以及生物檢測系統技術之發展。		
技術/產品應用範圍	生物,化學,生醫檢測分析及定量實驗之應用 酵素反應動力學之微量偵測		
技術移轉可行性及預期效益	此晶片系統為生醫之重要檢測工具, 預計可申請專利, 並發表期刊,同時投入產業界, 應用於日常生活中。		

註：本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。

國科會研究計畫結案報告

設計組裝及自動化控制微流體晶片平台在生化分析之應用

Design, fabrication and automatic control of microfluidic chip
platform for bioassay

計畫編號：NSC 101－2113－M－040－002－

計畫主持人：萬金鳳 助理教授

(中山醫學大學應用化學系所)

執行期間：101 年 08 月 01 日至 102 年 07 月 31 日

Research Project (研究計畫):

@設計組裝及自動化控制微流體晶片平台在生化分析之應用, (08/2012~07/2013, 國科會專題研究計畫, 主持人 萬金鳳, NSC 101-2113-M-040-002-)

研究計畫內容與研究成果

1. Development and Automation of Microelectromechanical Systems-Based Biochip Platform for protein Assay

Abstract

Miniaturized devices (lab-on-a-chip) for performing laboratory operations on microscale are appealing. Low sample requirement is one of the major advantages of these devices, therefore less costs is needed for running such platforms and also fewer wastes to be handled. Together with the unique behavior of liquids on microscale facilitating control of molecular diffusion and interaction makes miniaturized devices particularly useful in chemical synthesis as well as biological and/or chemical analysis. In this report, we designed a microfluidic platform with programmable microvalves capable to carry out routine operations. This platform was further optimized to contain universal sample-processing capabilities, using a three-layered hybrid PDMS–PDMS–glass structure. Precise programmable control of the volumetric flow rate can be achieved via the discrete digital control of fluids in pneumatically actuated microvalves. The specific protocols of the system are optical path platforms consisting of MEMS combined with photoresist arrays in microfluidic reactors for parallel biological analysis. To demonstrate the programming capabilities for biomolecular assay integration, we developed an automated assay with streptavidin immobilized on the photoresist patterned surface; these optical microfluidic platforms featured with a low sample requirement (0.5 μl per single assay) were then employed as a protein sensor, which has working concentrations ranged from 39.3 to 2500 nM for detecting biotin in the sample solution. The results suggest potential applications of these platforms in either routine assay purposes or specific applications such as high-throughput screening of protein-protein and protein-ligand interactions.

a. Bio manipulation platform – Microfluidic channels, microvalves and micropump have been designed, simulated and fabricated for chemical and biochemical reaction.

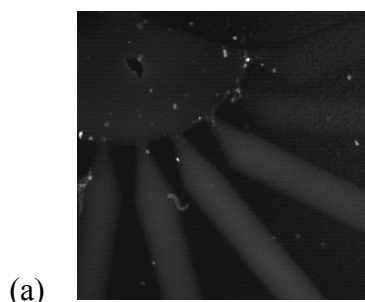


Figure 1. (a) Microfluidic chips & Microfluidic chip platform setup and the home-made microfluidic controller box.

b. microfluidic chip

A microfluidic chip includes a base layer, a fluid layer, and a gas regulating layer. The base layer includes a microarray detecting zone. The microarray detecting zone includes a substrate, a photoresist pattern layer, a blocking layer, a bonding layer, at least one linker molecule, and a probe molecule. The bonding layer is covalently attached to the photoresist pattern layer. The at least one linker molecule is covalently bonded to the binding layer. The probe molecule is covalently bonded to the at least one linker molecule for specifically reacting with an under-test molecule. The fluid layer is disposed over the base layer, and includes plural flow channels for introducing or collecting detecting reagents. The gas regulating layer is disposed over the fluid layer for controlling open/close statuses of the flow channels, thereby controlling a flowing condition of a fluid in the fluid layer.

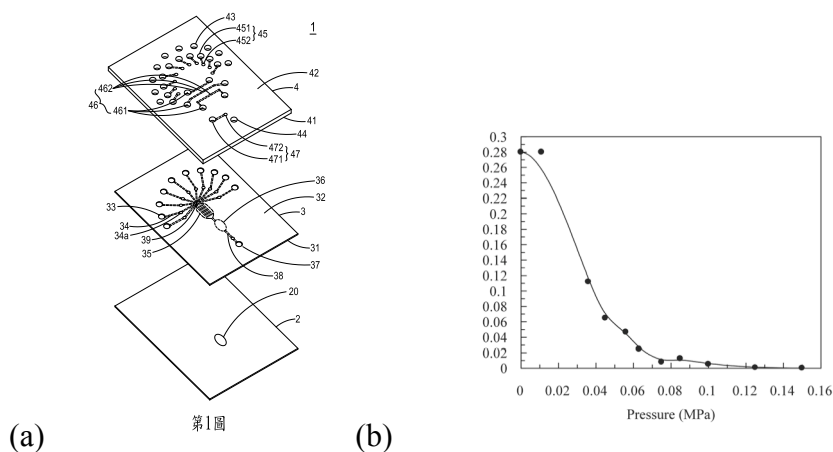


Figure 2. (a) Fabrication process on glass, (b) Microvalve closing vs applied pressure.

c. Simulation. Valve characteristics were first simulated under a wide range of conditions. We considered three major factors including (i) the width/height ratio of the microchannels, (ii) the

dimensions of the microchannels, and (iii) the PDMS thickness.

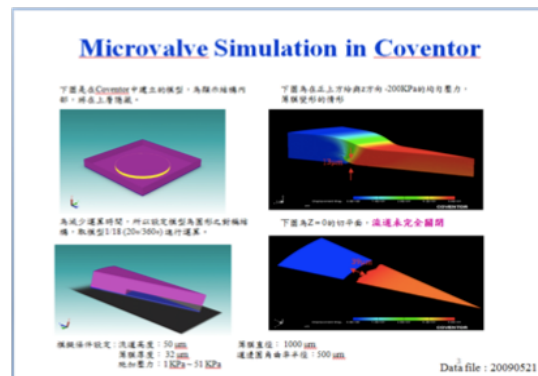


Figure 3. Computational model of a microvalve consisting of a $\pi \times 300^2 \times 100 \mu\text{m}$ control channel, a $\pi \times 300^2 \times 10 \mu\text{m}$ fluidic channel, and a 42- μm -thick membrane. (left) Undeformed configuration (right) Deformed configuration at actuation pressure.

d. A Virtual Mask Exposure System. A functional virtual mask MEMS-based projection system is set up. View interface and image arrays of 200 mm spots can be generated.

(a) 檔案太大, 刪掉圖檔 (b)

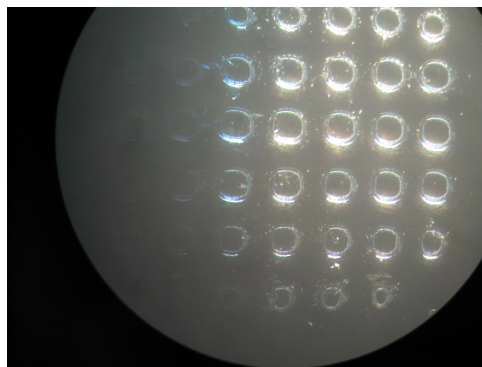


Figure 4. Optical path platform. (a) A Virtual Mask Exposure System. (b) Microarray patterns generated by our home-made virtual mask exposure system: arrays with spot size of 200 μm .

e. System integration – An user interface integrated image processing program and controller has been designed and demonstrated.

f. Biochip and fabricating method thereof

A biochip and a fabricating method thereof are disclosed. The biochip includes a substrate, a photoresist pattern layer formed on a surface of the substrate, a blocking layer formed on the surface of the substrate which is not covered by the photoresist pattern layer, a bonding layer covalently bonding

to the photoresist pattern layer, at least a linker molecule covalently bonding to the binding layer, and a probe molecule covalently bonding to the linker molecule for reacting with a molecule to be detected.

g. Initial system verification using biotin-streptavidin interaction is in progress.

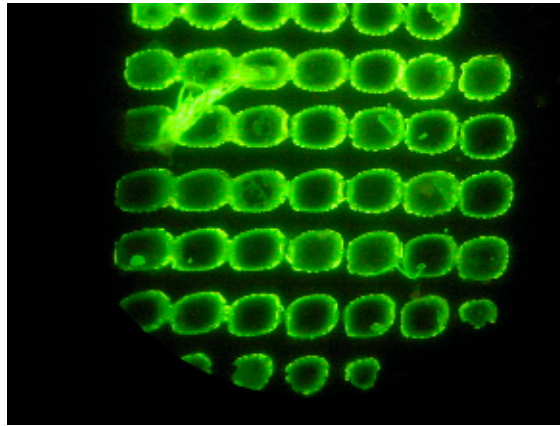
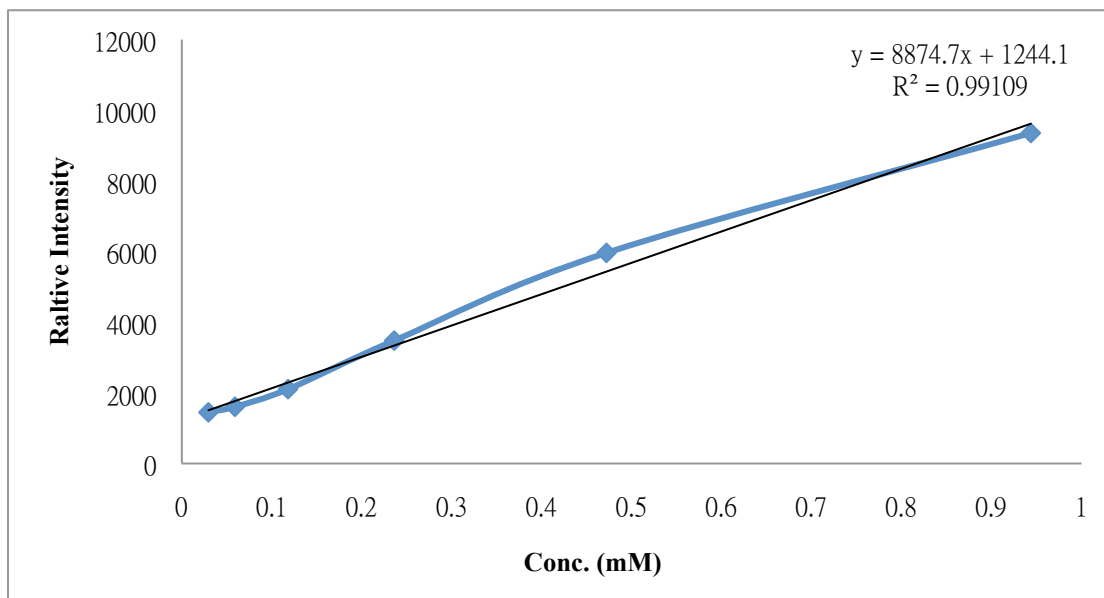


Figure 5. Microarray patterns generated by our home-made virtual mask exposure system: arrays with spot size of 300 μm . Magnetic Particles as Labels in Bioassays: Interactions between a Biotinylated Substrate and Streptavidin Magnetic Particles.

h. Fluorescence detection and data analysis. The fluorescent images were captured using a CCD camera cool SNAP HQ2 (Nikon, Tokyo, Japan) with a 400 ms exposure time. Relative fluorescence intensity was used to quantify the yield of biotin-4-fluorescein, which was calculated by scaling the intensity of the fluorescent particles to that of the reference area on the device. Data were analyzed by NIS-Elements BR410 Image analysis software.

The intensity values were calculated to average the intensity of each pixel for each spot. The density of the reactions was quantified by reporting the mean values of fluorescence density on a calibration curve, which was manually produced by spotting a dilution series of biotin-4-fluorescein on a glass substrate (0.5 μl /spot).

Relative Intensity versus Conc. (mM)



Conclusion

Advances in monolithic membrane valve technology have developed processing platforms for digital microfluidic assay automation. The programmability, routing, and low-sample volume requirements conferred by these systems offer significant advantages compared with conventional benchtop robotic laboratory automation systems. The digital transfer of fluids between microvalves in the automated microfluidic platform allows diverse serial and combinatorial sample processing operations on a microchip. The feature of programmability in this platform enables multiple applications within a single system which can be exploited to replace the specialized microfluidic circuits used in conventional lab-on-a-chip devices. Here we fabricated arrays of micro-pillars in the microreactor using an optical path platform with UV light, a lens and a DMD for the photopolymerization of an adhesive SU-8 substrate glued to treated glass slides. We demonstrated that the dimensions of the pillar and the quantity of the photopresist layers can be modulated by adjusting the patterning parameters. The photopresist microarray presented in this study was applied in protein immobilization; the identification of specific interactions with the immobilized protein can be achieved by detecting fluorescent moiety labeled on analytes. With the advantages described above, the automated microfluidic platform with an optical path reported here can be further exploited either to routine assay purposes or specific applications such as high throughput screening of protein-protein and

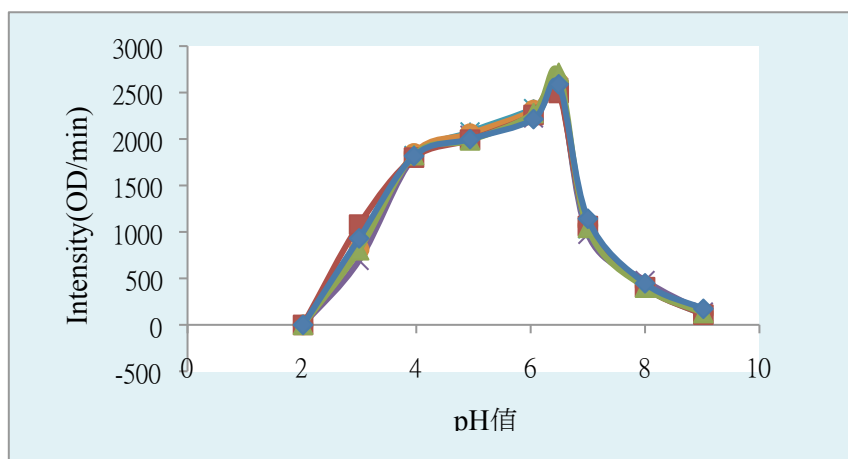
protein-ligand interactions.

2. Development and Automation of Microelectromechanical Systems-Based Biochip Platform for enzyme Assays

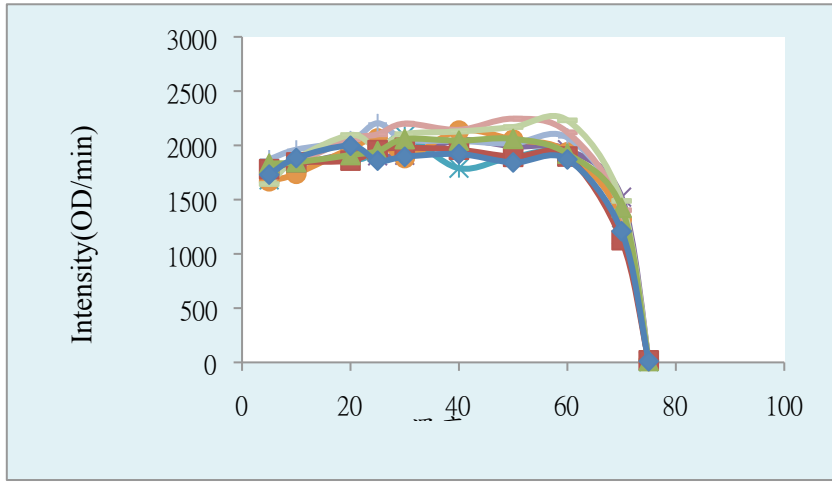
Abstract

The integration of biological assays and chemical reactions into microfluidic chip platforms has numerous advantages including automated sample processing, miniaturized analytical system, and sensitive detection onto a single microdevice. Moreover, the unique behavior of liquids on micro-scale allows better control of molecular diffusion and interactions. In addition to high through-put analysis, performing experiments on a micro-device will also reduce the cost of purchasing chemicals as well as the amounts of chemical wastes. This research includes the following three portions: (I) the development of UV optical path platform consisting of the core components of MEMS (Micro Electro Mechanical Systems), such as micromirror arrays and the light source, as well as its control for beam steering and accurate positioning of optical microarray; (II) the design, fabrication and automatic control of microfluidic chip platform, which could be used for parallel assays with multiple reagents to achieve multistep biological and/or chemical processes; (III) the integration of optical and microfluidic chip platforms as well as the automation of the entire system for performing high through-put biological and/or chemical processes on a microfluidic chip. This newly developed optical and microfluidic platform was utilized as a protein detection system by immobilizing glucosidase on photoresistant surface to quantify enzyme and enzyme kinetic in sample solution.

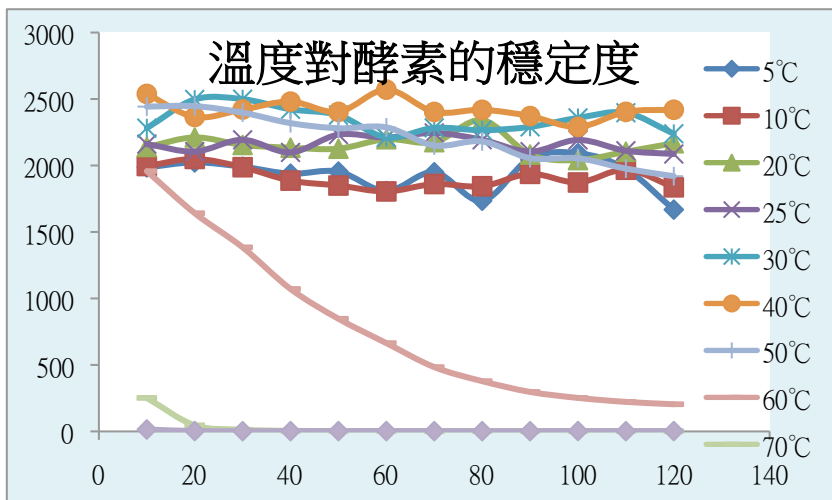
A. pH對酵素活性的影響



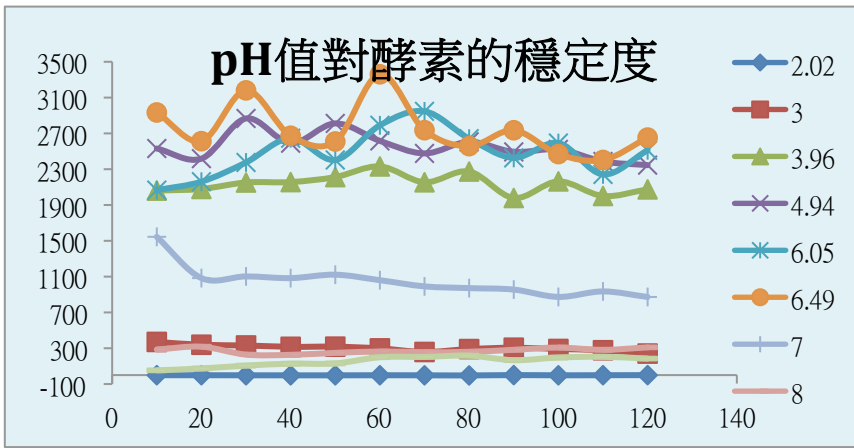
b. 溫度對酵素活性的影響



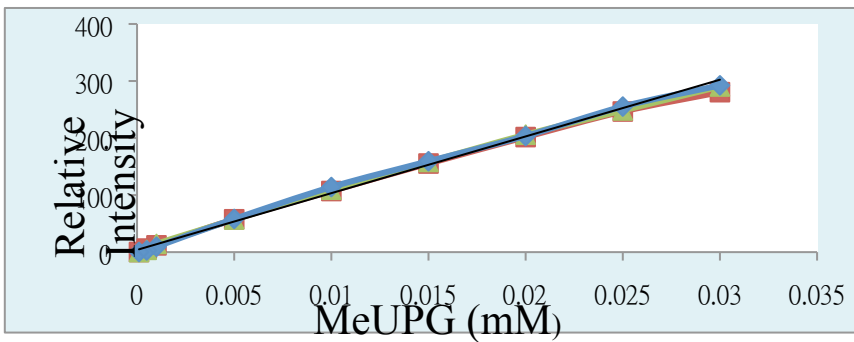
c. 溫度對酵素活性的穩定度



d. pH值對酵素活性的穩定度

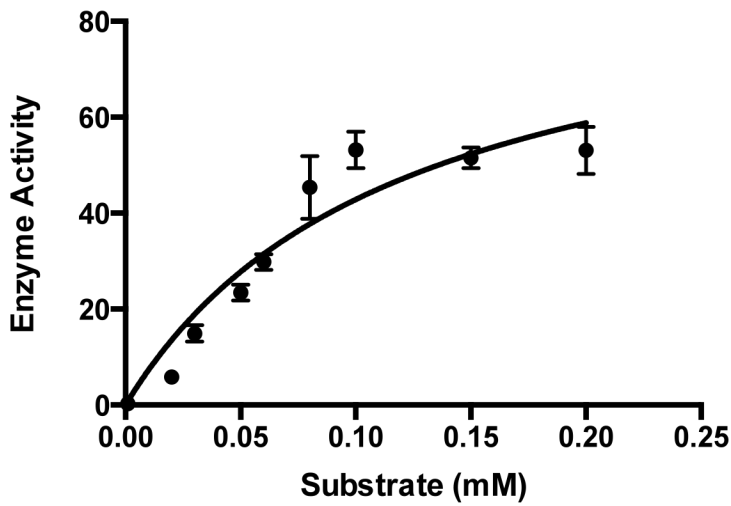


e. MeUPG vs Beta-glucosidase 定量實驗



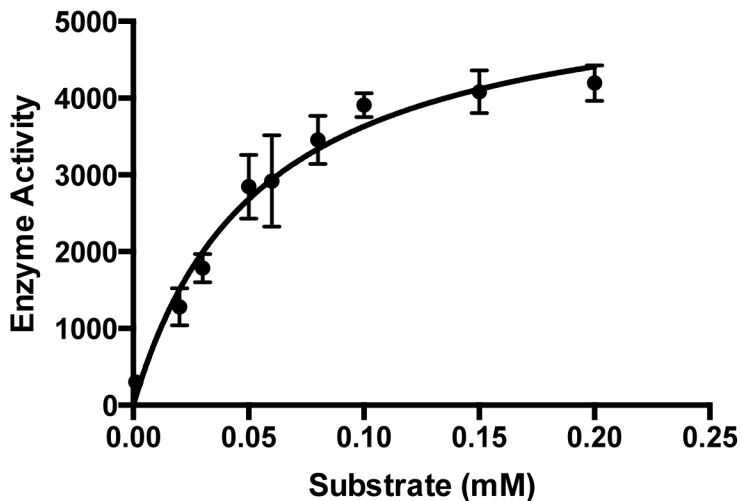
f. enzyme kinetic

Michaelis-Menten data (chip)



Vmax 93.81; Km 0.119

Michaelis-Menten data (instru)



V_{max} 5617; K_m 0.05472

Table 1. Comparisons of the microfluidic chip system and conventional fluorescence spectrophotometer

	Microfluidic chip	Fluorescence spectrophotometer ^a
Minimum sample volume (μ l)	0.5	3000
Working concentrations (nM)	39.3 – 2500	39.3 – 2500
Minimum sample requirement (fmole)	19.65	117900

^a Hitachi F-2500 fluorescence spectrophotometer

Conclusion

根據實驗結果，以溫度為變因， β -glucosidase活性在25~40°C有最大值，而溫度穩定度的探討，在25~40°C下放置120分鐘，活性不因溫度有明顯變化，因此在25~40°C下具有較平穩的穩定度；再以pH值為變因， β -葡萄糖苷酶活性在 pH值6.49有最大值，pH值穩定度的探討在pH6.49最佳。此外還做了在溫度25 °C & pH=6.52條件下，酵素對受質的定量實驗三次，發現有很好的再現性。

進一步探討酵素活性變化之特性，利用酵素動力學的方式，檢測 k_{cat} 和 K_m 。最後使用螢光儀和螢光顯微系統進行比較，螢光顯微系統比起螢光儀更可以降低使用藥品的成本並可降低化學藥品產生的污染。(實驗數據結果撰寫整理中)

研究成果

Patents (專利):

- 1 Chin-Feng Wan* (萬金鳳), (2013) Biochip and fabrication method thereof (生物檢測晶片及其製造方法), The Taiwan Patent, Application number 100130793, Application Date 20110826. [CxJxA=40x1x1=40](#)
- 2 Chin-Feng Wan* (2013) Microfluidic chip, The United States Patent, Application number PUS11302/811, Application Date Sep., 2012, Application number 13/632,784, Filing or 371(C) Date 10/01/2012, Publication Number US-2013-0096031-A1, Publication Date April 18, 2013~. [CxJxA=50x1x1=50](#)
- 3 Chin-Feng Wan* (萬金鳳), (2013) Biochip and fabrication method thereof, The United States Patent, Application number 13/560,711, Filing or 371(C) Date 07/27/2012, Publication Number US-2013-0053279A1, Publication Date Feb. 28, 2013~ [CxJxA=50x1x1=50](#)
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國科會補助計畫衍生研發成果推廣資料表

日期:2013/10/18

國科會補助計畫	計畫名稱: 設計, 組裝及自動化控制微流體晶片平台在生化分析之應用
	計畫主持人: 萬金鳳
	計畫編號: 101-2113-M-040-002- 學門領域: 感測器及微型分析系統
無研發成果推廣資料	

101 年度專題研究計畫研究成果彙整表

計畫主持人：萬金鳳		計畫編號：101-2113-M-040-002-					
計畫名稱：設計, 組裝及自動化控制微流體晶片平台在生化分析之應用							
成果項目		量化			單位	備註 (質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等)	
		實際已達成數 (被接受或已發表)	預期總達成數 (含實際已達成數)	本計畫實際貢獻百分比			
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	8	2	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	6	2	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力 (本國籍)	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	4	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	4	0	100%	件	
		已獲得件數	1	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力 (外國籍)	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

<p>其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)</p>	1
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	成果項目	量化	名稱或內容性質簡述
科 教 處 計 畫 加 填 項 目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與(閱聽)人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以 100 字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文： 已發表 未發表之文稿 撰寫中 無

專利： 已獲得 申請中 無

技轉： 已技轉 洽談中 無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

本研究結合微機電系統工程、化學合成技術及生物化學以及生物科技之基本知識，對於未來科技研究工作之發展甚具潛能，開發之自動化晶片平台及光學光路平台可申請專利並兼具論文發表之學術性。生物晶片應用在疾病檢測、醫藥的篩選等對於未來之生物醫學將有莫大之幫助。

本計畫在學術研究方面之具體發展為：

- 本計畫所研發之微流體晶片系統可應用於醣類化學之合成，例如抑制劑的合成。
- 本計畫所研發之微流體晶片系統可應用於醣類酵素之鍵結，以製作蛋白質晶片。
- 本計畫所研發之檢測系統，可用於檢測醣類酵素與抑制劑分子接合過程。
- 本計畫所研發之微流體晶片系統可應用於藥物之開發。
- 本計畫所研發之微流體晶片系統可結合本校醫學大學之特色，應用於生醫分子檢測。

預期可發展之相關技術包含有半導體製程技術之應用、微系統加工技術研發、微結構之析設計、醣類合成化學在微系統之應用、蛋白質體與微系統之整合以及生物檢測系統技術之發展。此微流體晶片為生醫之重要檢測工具，預計可申請專利，並投入產業界，應用於日常生活中。