行政院國家科學委員會專題研究計畫　成果報告

HHHM 核酸/蛋白質微陣列之研發-生醫檢測用微井陣列晶片

計畫類別：整合型計畫
計畫編號：NSC91-2323-B-007-004-
執行期間：91 年 08 月 01 日至 92 年 07 月 31 日
執行單位：國立清華大學電子工程研究所

計畫主持人：黃瑞星

報告類型：完整報告

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中　華　民　國 92 年 11 月 7 日

製藥與生物技術國家型計畫年度研究成果報告

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※ HHHM 核酸/蛋白質微陣列之研發 ※
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計畫主持人：黃瑞星　教授

中　華　民　國　九十二年　十　月　三十　日

製藥與生物技術國家型計畫年度研究成果報告

HHHM 核酸/蛋白質微陣列之研發– 生醫檢測用微井陣列晶片

The Development of HHHM DNA/Protein Microarray –Micro-well Array for Biomedical Applications

計畫編號：NSC 91-2323-B-007-004

執行期限：91 年 8 月 1 日至 92 年 7 月 31 日

主持人：黃瑞星 教授 清華大學 電機工程學系

計劃參與人員: 陳建亨 陳彩芬 林直慶

一、中文摘要

本子計畫旨在利用微加工技術設計和製作一個微井陣列晶片基板作為雜交微容器，此晶片亦將擁有一些特殊功能以便執行 HHHM 的程序，集積於此晶片的特色功能將包括微井陣列、微加熱器、溫感器、簡單訊號處理，以及振動 / 擾動，此中一些功能如溫控器在初期將用零件組成，最後目標是把所有功能集積在單一晶片上供生醫應用。

關鍵詞：微井陣列晶片、微加工技術、生醫應用

Abstract

This subproject is to design and fabricate a micro-well array chip base as the liquid hybridization micro-container by micromachining technique. The chip also will have some built-in special function in order to execute the HHHM process. The features integrated on the chip will include micro-well array, micro-heater, temperature sensor, simple signal processing, vibration/ stimulation.

Keywords: Micro-well array chip, HHHM process, hybridization

二、緣由與目的

As the Human Genome Project gets towards completion of the first finished human sequence, DNA chip technologies offers the potential to open wide new windows into the fundamental research of life science as well as clinical applications. There are many critically important questions about this new field both in the instrumentation and the biology aspects those are yet un-addressed. One of the problems of the existing microarrays is the sensitivity, especially, when apply the microarray techniques in the study of human biopsy. The other deficiency of the existing microarrays is that they can only be used for DNA only. In this proposal, we will develop and construct a DNA/Protein Microarray system. The new kind of microarray, HHHM microarray, produced by our system can be used both for DNA-DNA hybridization, protein-protein hybridization and DNA-protein hybridization. Besides, the variety of the capable hybridized material, compare to the Brown’s type DNA microarray, the HHHM microarray has the following advantages:

1. No need of coating treatment.
2. Easier isolation and purification of probes.
3. Improved sensitivity.
4. Reduced processing produce.
5. Easier detection (scanning) method.
6. Easier printing method (no surface contact).
7. Reusable chip

This subproject is to design and fabricate a *micro-well array* chip base as the liquid hybridization micro-container by micromachining technique. The chip also will have some built-in special function in order to execute the HHHM process.

The features integrated on the chip will include micro-well array, micro-heater, temperature sensor, simple signal processing, vibration /stimulation. Some of the functions, for examples, temperature controller will be developed using discrete components in the first year and eventually aimed to be integrated such that all the necessary functions are in a single chip for biomedical applications.

三、結果與討論

(1) Fabrication of micro lens and testing of micro-well array chip provide multi-function for liquid phase biomedical processing and detection:

We develop a novel micro-well array chip fabricated by micromachining and CMOS technology for biomedical application. The chip is designed to provide multi-function for liquid phase DNA or protein sample processing and detection, its function and structure includes heating, cooling, temperature sensing, vibration agitation, control circuits, array of micro-wells, and a transparent cover with liquid seal at each well. The embedded heater shows good linearity and can heat up to 101 ℃ with ~20mA current supply, liquid phase biomaterials such as probes and samples can be processed (hybridization, mixing, separation, washing, and detection) on the chip.

Figure 1 shows the temperature dependent of micro-well under different excitation currents. The embedded heater shows good linearity and can heat up to 101 ℃ with ~20mA current supply. The dynamic characteristics of the heating/cooling process are shown in Figure 2.

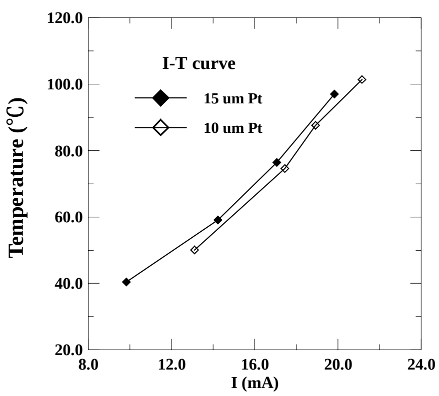


Figure 1 The temperature dependent of micro-well under different excitation currents

In the heating process, the temperature change takes about ~25 seconds from 50 to 90℃ with ~20mA constant current heating. In the cooling process, the temperature change takes only about ~5 seconds from 95 to 50℃. This fast heating/cooling rate is sufficient for the thermal cycling of liquid phase process controls, e.g. PCR [1-2].

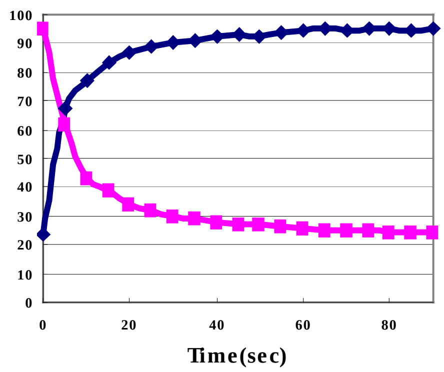
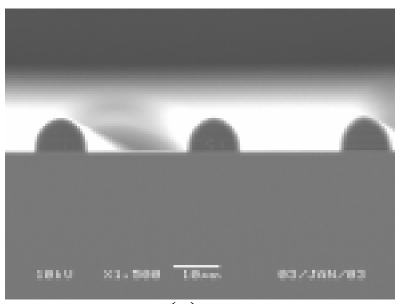


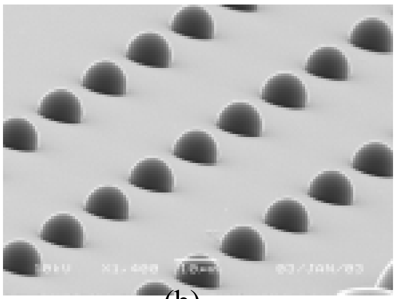
Figure 2 The dynamic characteristics of the heating/cooling process

The photo-resist, AZ 4533 and AZ4563, were used to fabricate the micro lens. On a 7740 glass, the resist was coated by using spin coater and patterned by lithography process. After that, the sample was heated up to 150℃ for 2 min. Then the resist re-flowed and become the micro lens after cooling down.

Figure 3(a)-(b) show the cross-section and top view of 5-10 um micro lens and Figure 4(a)-(b) show the 50-100 um ones. As figure 3-4 shown, the micro lens can be fabricated by using resist re-flow process successful. The maximum diameter of micro lens is about ~500um in our experiment.

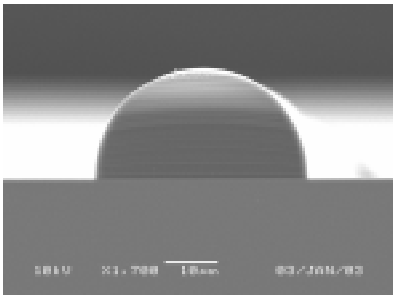


(a)

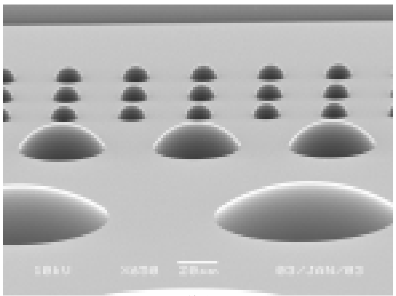


(b)

Figure 3(a)-(b): the cross-section and top view of 5-10 um micro lens.



(a)



(b)

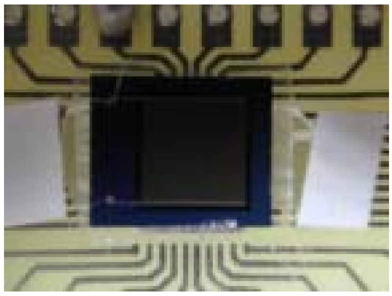
Figure 4(a)-(b): the cross-section and top view of 50-100 um micro lens.

(2) Testing and Theoretical approach of micro TE cooler by surface micro-machining technology:

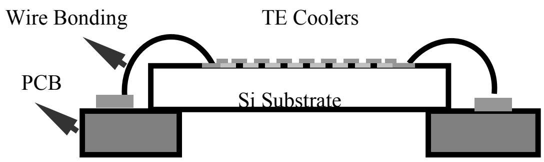
We developed a novel on-chip integrated poly-Si TE (thermoelectric) cooling device fabricated by surface micromachining technology. The area of the bridge type Peltier [3-4] element is about ~40×40 (um^2) and there are about ~62,500 elements in 1 (cm^2) chip area.

We have developed a novel integrated poly-Si TE cooling device fabricated by surface micromachining technology. The typical fabrication process is illustrated in Figure 6. First, the SiO_2 of 500 nm was grown and a LPCVD SiN_x of 300 nm was deposited on 4-inch (100) Si wafer. Second, the PECVD sacrificial oxide about 3um was doped and patterned. Then, the structure layer, poly-Si, about 1.5um, was deposited, diffusion, and patterned. The contact layer, Au/Cr = 500nm/50nm, was deposited by DC-sputter and patterned by lift-off. After the fabrication, the device was released in 1: 10 diluted HF. There are two kinds of TE devices fabricated in our experiment.

The Figure 5(a)-(b) show the photograph and the cross-section of the testing package. The chip is placed on the PCB board that has a drilled hole under the chip and the TE coolers are connected to PCB board by wire bonding.



(a)

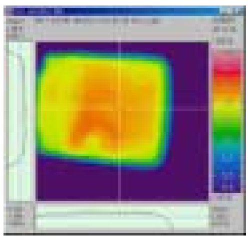


(b)

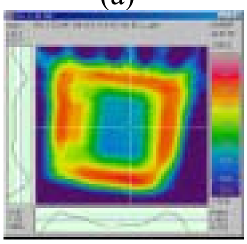
Figure 5(a)-(b): the photograph and the cross-section of the testing package.

Figure 6(a) show the IR images of the TE device before sacrificial oxide release, while Figure 6(b) shows the images of the device after sacrificial oxide release, under various current driving. The regions of TE coolers (the temperature is T_{cooler}), Si substrate (T_{sub}), and PCB (T_{pcb}) are also indicated in Figure 6(b). Under high current driving, the samples with sacrificial oxide releasing, as shown in Figure 7(b), had more clear thermal images (and/or easier to define the regions of TE coolers, Si substrate, and PCB) than that the samples without releasing, as shown in Figure 6(a).

This indicates that the parasitic thermal conduction effect (thermal bypass) may occur with oxide (without oxide releasing) and can be minimized after oxide releasing. As the figure shows, under 80 mA current drive, the temperature value of the TE device region is ~5 ℃($\Delta T=T_{sub}-T_{cooler}$) lower than that of Si substrate; ~ 15 ℃($\Delta T=T_{pcb}-T_{cooler}$) lower than that of PCB.



(a)



(b)

Figure 6: IR images of the device (a) before sacrificial oxide release and (b) after oxide release under current drive

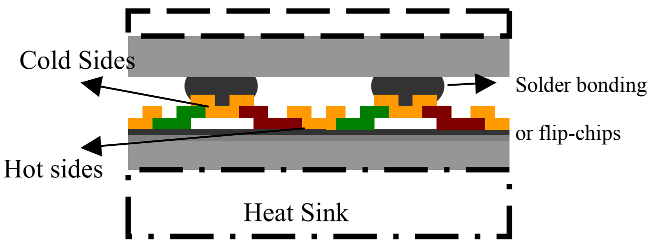


Figure 7: The possible application of the novel poly-Si TE cooler

The possible application of the novel poly-Si TE cooler is shown in Figure 7. The Si substrate, which is connected to the hot metal sides, can be bonded to the heat sink. The cold metal sides can be contact to the heat load/objects, for example, CMOS ICs, which need to be cooling, by using the solder bonding or the flip-chip.

Although theoretical approach indicates that Z could improve greatly by using the oxide sacrificial releasing process, a realistic estimation of the achieve ΔTmax should be compared with measurement results. For poly-Si the best obtainable values are: αn-αp ~350 μV/K, λn~λn~20 W-1mK-1, ρn=8 μΩm, and ρp=28μΩm. Calculation of the maximum achievable temperature difference using this data gives: ΔTmax = (1/2) ZT ~ 11 K.

This is a theoretical value and does not take into account effects, such as thermal leakage and Joule heating at Au-Poly electrical contacts. The cooling performance of structures should be significantly reduced by the contact resistance. In our samples, the total resistance is about 29.5-32.2Ω which was measured by HP4156. The poly-Si lines are ~16 μm long and ~8 μm wide.

The poly-Si thickness is 1.5 μm. By combining this information with the doping concentration given above, results in a total line resistance of about ~20Ω. The Au-n-poly plus the Au-p-poly contact resistance is very like to near/ exceed ~10 Ω. The Joule heating of the cold junction causes a reduction of the maximum achievable temperature difference.

That is roughly proportional to Rcontact/Rline ~ 50%. Therefore, ΔTmax is limited to 6 K. This is near to the temperature difference between TE cooler and Si substrate (Tsub-Tcooler ~ 5K).

四、成果發表

此項研究相關結果，總計有一項專利申請中、二篇國際研討會論文已發表、一篇國際性期刊論文已接受和一篇國際性期刊論文審查中。茲列於下:

[1] 生物晶片掃描裝置，申請案號 90221724, 中華民國

[2] “ A Novel Micro-well Array Chip for Biomaterial Processing and Detection in Liquid Phase,” Pacific Rim Workshop on Transducers and Micro/Nano Technologies, 2002.

[3]”A Poly-Si Thermoelectric Cooling Device Fabricated by Surface Micromachining Technology,” Transducer’03, The 12th International Conference on Solid-State Sensors, Actuators and Microsystems, 2003.

[4] “ A Novel Micro-well Array Chip for Liquid Phase Biomaterial Processing and Detection, ” to appear in Sensors and Actuators (2003).

[5] “Characterizations of Novel Poly-Si Thermoelectric Cooling Device Fabricated by Surface Micromachining Technology,” submitted to IEEE Trans. on Electron Devices.

五、參考文獻

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[2] Thomas Laurell, et al, Protemics—protein profiling technology: the trend towards a microfabricated toolbox concept, trends in analytival chemistry 20 (2001) 225-231.

[3] D. D. L. Wijngaard, et al., Sensors and Actuators 85(2000) 316-323.

[4] Marc Strasser, et al., Transducer ’01 Eurosensors XV (2001).