Regular paper http://dx.doi.org/10.13067/JKIECS.2013.8.12.1875

DNA 마이크로어레이 프린팅을 위한 사용자 인터페이스 적용기술

박재삼*

Implementation of User Interface for DNA Micro Array Printing Technology

Jae-Sam Park*

요 약

마이크로 어레이 기술은 유전자 네트워크의 순서 와 게놈의 통합과 같은 많은 업적을 기여하고 있으며, 이러한 기술은 유전자 발현의 패턴을 조사하기 위한 수단 등으로 잘 확립 되어있다. DNA 마이크로배열은 Affymetric 칩을 이용하여 대량의 DNA 서열을 합성 할 수 있는데 기존의 DNA 어레이 스포팅에는 일반적으로 접촉방식과 압전전자 방법등 두가지 유형이 있다. 접촉방법은 유리 슬라이드 표면과 접촉하도록 스포팅핀을 사용하는데 이 방법은 표면 매트릭스의 손상이나 상처가 발생할 수 있어 단백질이 오염 되거나 특정 결합을 방해할 위험이 있다. 반면에 압전전자 방법은 대량 생산이 가능함에도 불구하고 결과를 인쇄할 분석기가 필요하므로 현재 실험실 내에서만 수행 가능한 실정이다. 본 논문에서 유리 슬라이드 표면에 닿지 않고 지속적으로 일관성 있게 스포팅이 가능하도록 하는 진보된 방법을 제시한다.

ABSTRACT

Micro-array technology contributes numerous achievements such as ordering of gene network and integration of genomic. This technology is well established as means for investigating patterns of gene expression. DNA micro-arrays utilize Affymetric chips where a large quantity of DNA sequences may be synthesized. There are two general type of conventional DNA array spotter: contact and piezoelectric. The contact technology used spotting pin technology to make contact with the glass slide surface. This may caused damage or scratches to the surface matrix where protein will be contaminated and may not bind specifically. Piezoelectric technology available at this present time on the other hand requires the analyzer to print the result that can only be done within the laboratory despite of mass production. Therefore, in this paper, high-throughput technology is developed for providing greater consistency in feature spot without touching the glass slide surface.

키워드

CoreChart Software, Deoxyribonucleic Acid (DNA), Micro Array Spotting, Piezoelectric
코어차트 소프트웨어, 데옥시 리보 핵산(DNA), 마이크로 배열 스포팅, 압전전자

Ⅰ. INTRODUCTION

Several DNA Micro array[2,10] printing technologies has been developed since few years ago for the use in biomedical industry. One such is the Mechanical Micro-Spotting technology where pre-customized sample is loaded into a spotting pin by capillary action onto the glass slide surface. Figure

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접수일자 : 2013. 10. 01 심사(수정)일자 : 2013. 11. 25 게재확정일자 : 2013. 12. 16

1 shows this mechanism. Although this printing technology allows transfer of pre-customized oligonucleotides onto a solid surface, however the lack features of this method are the mass production issue and failure on producing uniform spots consistently. Hence, the recent developed ink-jet dispensing technology[14] has been taken into consideration in producing a flexible DNA[11,12] Micro-array[15] Printing Machine for pre-customized oligonucleotides with the aim of non-contact, low cost, flexible, high throughput, repeatable, rapid implementation and yet easy to use. For these purposes, a commercially available ink-jet printer, Epson R-230 has been selected[6]. This printer offers the piezoelectric inkjet printing technology (On demand inkjet). By using this technology, droplets are mechanically evicted from a nozzle in an ink chamber when a piezo-crystal is activated by current impulse. Figure 2 shows the piezoelectric printing technology.

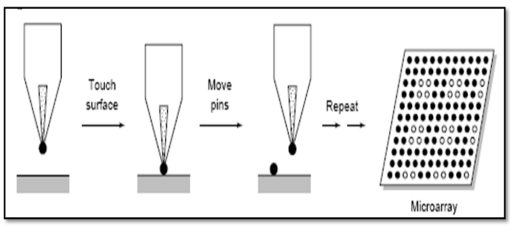

그림 1. 마이크로스포팅 프린팅 메커니즘
Fig. 1 Micro spotting mechanism(schena, heller, theriault, konard, lachenmeier and davis(1998)

The DNA slide glass tray is proposed to replace the CD tray printing facility. Three main sections have been carried out in this paper mainly are the motion control system, mechanical design as well as the user interface from the computer to printer via the implementation of microchip PIC16F877. Due to broad coverage of the paper, user interface for the DNA printer will be emphasized in this paper.

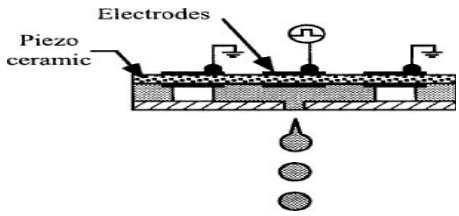

그림 2. 피에조일렉트릭 프린팅 기술
Fig. 2 Piezoelectric(push mode) printing technology (lee 1998)

II. PROCESS DESCRIPTION FOR DNA MICRO ARRAY PRINITNG TECHNOLOGY

2.1 DNA Micro Array Printing Process using Piezoelectric Technology.

Firstly, phosphoramidite is sprayed on the glass slide surface. Next Acetic Anhydride & N-methylimidazole reagent is used to Capp the whole slide of the reaction column. This is to make sure all spots on the glass slide are uniformly capped. After that, washing process will be performed to de-Capping (remove) the excessive phosphorus chemical that was sprayed on previous step before continuing on building the DNA chains on the reaction column followed by the drying process. Lastly, Tetrazole will be added to the next base monomer before the second layer of phosphoramidte chemical would be released. This process repeats until the pre-synthesized oligonucleotides are obtained. Figure 3 summarize the process steps on the printing process.

2.2 Printing Process For Flexible DNA Micro Array Printing Technology.

The teams have produced a depiction of DNA micro-array printing process that would result upon completion of this paper. In essence, DNA printing will only start once the printer has initialized the glass slide on the CD tray. Instruction and data input will be sent to the hardware via the Visual Basic Programming Language in tandem. Once the

CD tray (glass slide tray) and sample have been detected, for first layer of printing, phosphoramidite will be sprayed on the glass slide. Followed by the capping printing, where two reagents will be used to avoid the contamination of protein[1]. Washing printing will be done to ensure the extra acid or solutions are removed. De-blocking or de-capping will be the next step to ensure reaction column on the surface is activated to build oligonucleotide chains. These processes continue until the customized synthesized oligonucleotides are obtained.

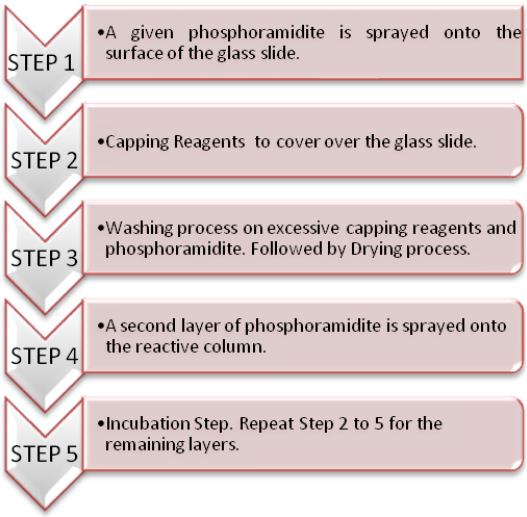

그림 3. DNA 마이크로어레이 프린트 진행과정
Fig. 3 Summary of DNA micro array printing process

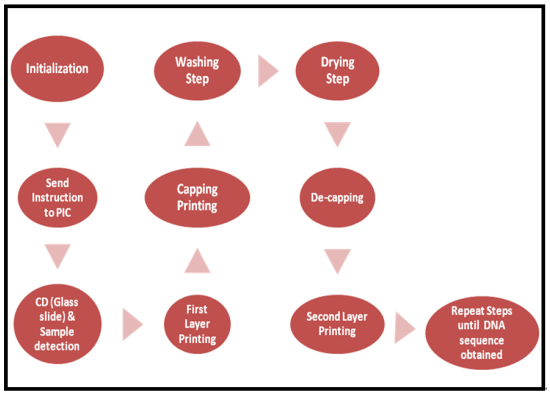

그림 4. DNA 마이크로어레이 프린트 진행과정
Fig. 4 Summary of DNA micro array printing process.

III. PROCESS DEVELOPMENT OF FLEXIBLE DNA MICRO ARRAY PRINTING MACHINE

3.1 Motion Control System Module.

The motion control of the printer has been divided into three main modules which are the x, y and z direction, namely left & right, backward & forward and upward & downward movement respectively. Fundamentally, this motion control modules are built by two DC motors and one stepper motor. The functions of the DC motors are to control the height and movement of the print head. While Stepper motor is used to feed the CD tray mechanism. Sensors play a crucial part as it provides feedbacks to the controller and print head positioning. Three sensors have been detected in this printer. Encoder strip sensor, CD tray sensor and Height sensor with respect to the stepper motor.[16,17,18]

3.2 Mechanical Mechanism System Module

Due to time constraint, the teams have not yet reached the implementation of the mechanical design. However, the preeminent mechanism design alternative will be illustrated here. Uni-Graphic Software has been used to produce this mechanical design. First 5 demonstrate that washing and drying mechanism is to be arranged at the back of the printer. Hence, once printing and capping procedure are done, the sample would be sent to the back of the printer for washing and drying. However, due to the issue on space and chemical wastage, an improvement has to be done.

Second stage of design exemplifies the design mechanism where space of the printer is fully utilized. Front part of the printer will be used for the washing and drying process. Besides, chemical waste tank would be built underneath of the printer. This will save the space of printing process and cost purchasing nitrogen gas as this printer shall be encapsulated in nitrogen gas environment

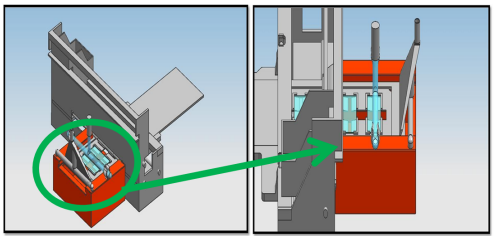

그림 5. 초기에 설계된 세척과 건조 장치
Fig. 5 First design on washing & drying mechanism

to prevent reaction between DNA solutions and environment moisture.

The DNA micro array glass slides tray is determined to be placed in parallel to each other. This is to ease the printing process because Capping, De-capping, Washing and Drying printing has to be taken into consideration in the later stage of project continuation. In summary, it can avoid the contamination during printing process, increase the productivity of printing, reduce the chemical usage and save the cost of operation.

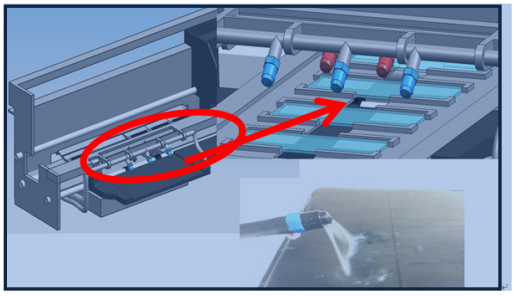

그림 6. 두번째 설계된 세척과 건조 장치
Fig. 6 Second design on washing & drying mechanism

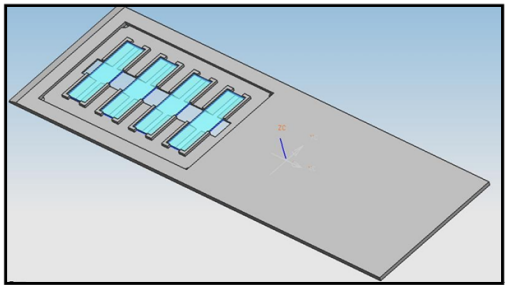

그림 7. 설계된 유리 밀판
Fig. 7 Glass slide tray design

3.3 User Interface System

The User interface was created using Visual Basic. Visual Basic programming acts as the communication medium where it sends the data information from the Computer to PIC16F877 microcontroller in association with the CoreChart software[7]. Command functions for DC and stepper motors have been set in the W parameter of the CoreChart Software. This PIC is then integrated with an USB programmer PIC16F745[9,13]. It incorporates as the USB interface[3] to the printer. PIC16C745 was implemented due to its ability as Microchip's[4] low speed USB microcontroller to facilitate USB communication between the computer and printer[5]. The most significant features of this microchip is to provide lowest level interface to the USB. The host and printer communicate in forward direction while signaling rate from the computer to printer is Full speed: 12Mbps. For instant, user will enter the command and data information from the user interface and send the data to the PIC16F877 microchip controller.

The data will then stored in the PIC chip before it is sent to the printer. Once the data has been received, the printer will perform the task instructed by the user. Figure 8 depicts the process between the host and DNA printer.

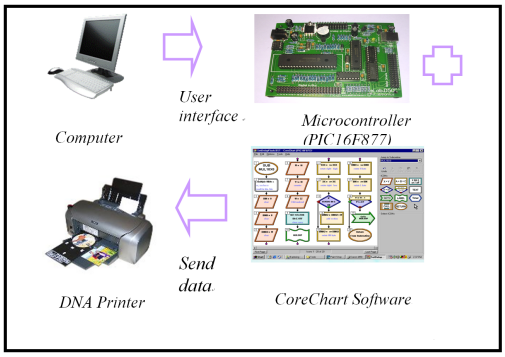

그림 8. 컴퓨터와 DNA 프린터 연결 및 프린팅 진행 과정
Fig. 8 Process overview between computer and DNA printer

In summary, once the USB standard protocol has been serviced, PIC16F877 will receive commands and data from the host. It will then transmit the requested data to the hardware by translating ASCII commands into actions.

3.4 User Interface Explanations

The user interface that has been created illustrates three main frames. First frame was designed to control the Printer Head. When user clicked on the Move Right or Move Left button, this VB interface associates with the parameter w = 1 and 2 in CoreChart respectively. Distance in this frame is to define the distance size for the height leveling of the DC motor. Encoder sensor will sense the amount of encoder strips (transparent strips in the printer) to perform the distance movement of the printer.

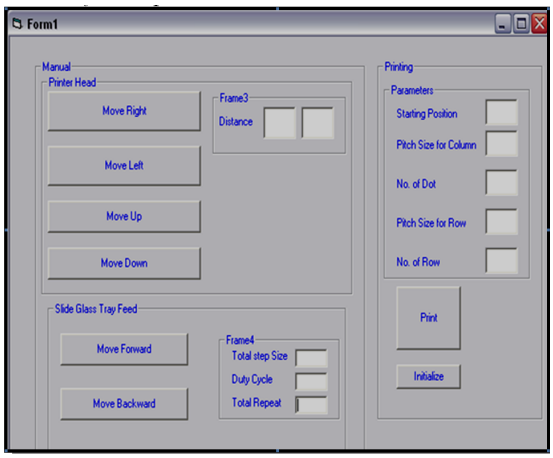

그림 9. 비주얼베이직으로 제작한 사용자 인터페이스
Fig. 9 Second user interface implementation using VB

The user interface Second frame is Slide Glass Tray Feed describes the movement of the stepper motor in forward and backward direction. User will enter the amount of steps to be moved and time of delay with respect to the Pulse width Modulation in the stepper motor. The total repeat is referred to the amount of dots to be printed in row.

Thirdly, in the Printing frame, the user will be asked to enter distance of position for the printer to perform the task. Pitch size refers to the distance of dots that have to be printed out as well as distance between the amounts of rows for oligonucleotides sequence.

Print button will then send the command to the hardware and instruct the printer to perform the task where data and command are mainly fetched through the Printing Frame.

Initialize button on the other hand will drive the DC and stepper motors manually. The data was obtained from the Frame in Printer Head and Slide Glass Tray Feed. In summary, printer head will move up by the amount of distance entered by the user. Then, it will perform the home positioning before moving the print head down. Once height initialization is performed, it will start printing. Lastly, it will eject the glass slide tray out from the printer. Figure 10 summarize the printing process that will performed by the printer.

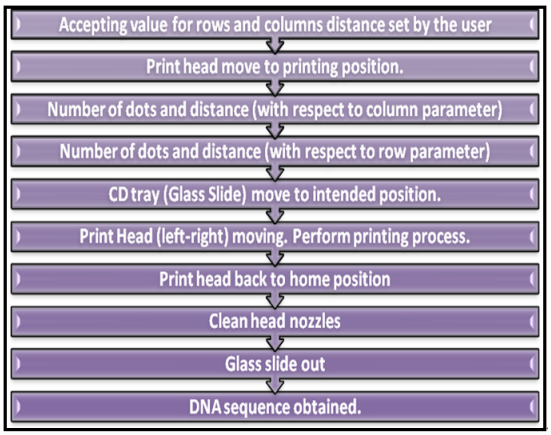

그림 10. DNA 시퀀스 프린팅 과정 요약
Fig. 10 Summary of printing DNA sequence

IV. RESULT AND DISCUSSION

Due to time constraint, the print head firing sequence has not been discovered. With six

cartridges offered by the printer and 90 nozzles for each of the cartridge, the exact pin used for firing in each cartridge was not discovered. Therefore, the teams have used the "marker ink" as the nozzle head by placing it beside the cartridge holder to perform the printing demonstration. Data entered for this printing job is **Pitch size : 50, Dots : 7, Total Step size: 30, Duty cycle: 1 and Total repeat : 4 rows.** Results of testing were shown in figure 11.Different voltage has been applied from 8V to 13V. In every 1Volt of increment the values of distance between dots varied for 0.5mm.

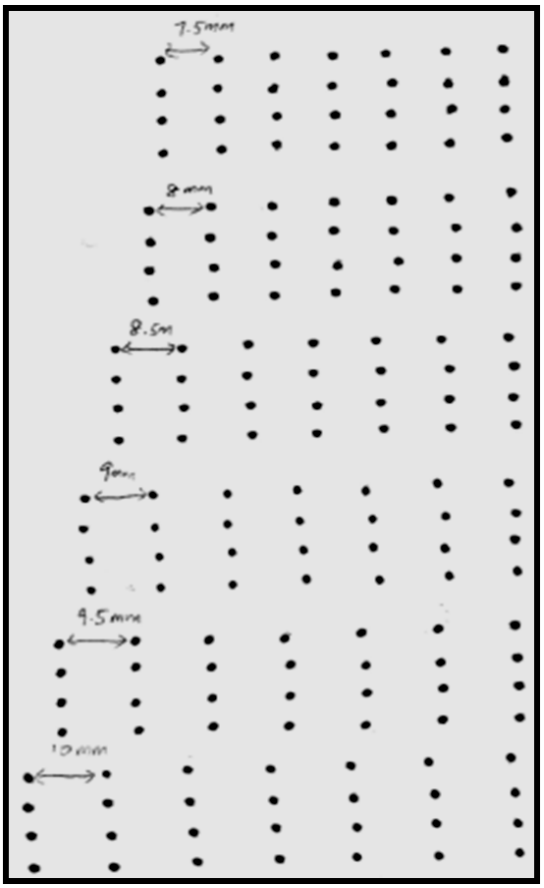

그림 11. 실험 결과
Fig. 11 Experiment result

On the whole, once the DNA micro-array printer is plugged in to the computer, the host will immediately enumerate the printer. The first function called is the HID_GetHidGuid in order to get the GUID (Globally Unique Identifier). Function "CByte" is used to return an expression variant of type Byte. For example, when users enter the single-precision, double precision or integer arithmetic type of data, it will return the data or value to byte. Public Subroutines such as the WriteToDevice, ReadReport and DoEvents eliminate the need for a separate openFile function in the CoreChart Software. Besides that, it ensures the printer is connected to the computer. If the device is not found, it will run through the process and find it until printer is detected. ReadReport on the other hand returns a buffer from the printer. This function will immediately return the oldest buffer from the printer to computer. WriteToDevice will send an 8 byte command buffer to the printer. Command is passed as a string type. Internally, WriteToDevide copies the string over to an array of 8 bytes. For Command4 in the Visual Basic programming, when W=2 in CoreChart software, print head will move down for two second. This shows that W=2 is set to print head for down movement function. Stepper motor was set to 3. User can feed the glass slide tray by specifying step size of the stepper motor. With respect to encoder sensor, DC motor move from the right to left direction according to the amount of strips (data information). Printer will then start printing the DNA spots.

Lastly, in getting familiar with the mechanism and features offered by the printer, the teams have performed experiments on determining the smallest size of the dots that can be produced by the Epson printer. AutoCAD software was used to obtain the results. In conclusion, maximum size of visible diameter to our rough eye is 0.05mm.

Hence, evidence has proved that this EPSON

printer is not only able to provide ink jet dispensing technology, it also provide constant dot of matrix. More than that, once the prototype was successfully developed, it will accomplish the objective of producing a flexible yet economically available DNA printing machine.

표 1. 전압레벨에 따른 도트간 거리
Table 1. Results for distance value with different voltage

Voltage Applied	Distance between dots:
8V	7.5mm
9V	8.0mm
10V	8.5mm
11V	9.0mm
12V	9.5mm
13V	10.0mm

V. CONCLUSION

Figure 12 shows the printing process for the EPSON R-230.

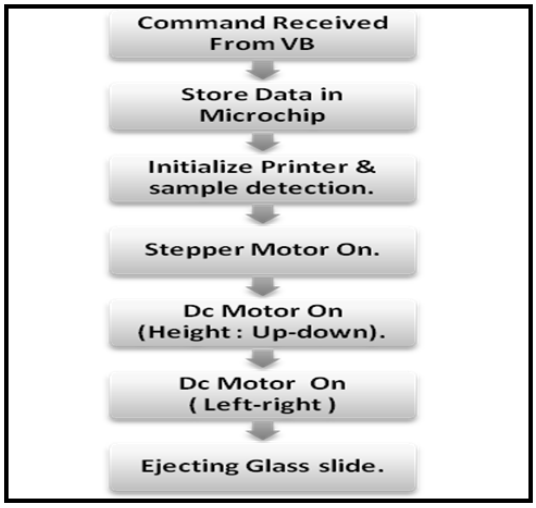

그림 12. 엡숀프린터 프린팅 과정
Fig. 12 Epson printer printing process

With the several DNA Micro Array printing technologies offered in the biomedical industry, ink jet dispensing has to be developed to produce high throughput of the result. This DNA micro array printing machine will be successfully implemented once the research and development on firing sequence is completed. In the future, specific software to print the oligonucleotides will be developed and further investigating on the contamination issues between the chemical reagents and glass slide design. On top of that, issues for drop size, speed and force can be controlled accurately on voltage variation.

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