Collaborative Efforts between the Local Industry and Engineering Technology and Biology Students in Building a DNA Microarrayer

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Abstract

The current paper describes the collaborative efforts between engineering technology students, biology students, and a local industry in building an advanced microarrayer system for DNA testing. BioVentures, a biotech company located in Murfreesboro, TN and one of the world largest suppliers of DNA markers has sponsored the project. BioVentures has provided all materials and support while a combination of graduate and undergraduate student team from MTSU built the system and tested it. The team was supervised by a team of faculty from both the engineering technology and biology departments as well as biotech engineers from BioVentures. These collaborative efforts have resulted in a very positive and promising experience for all parties involved. A brief account of this experience and its outcomes, especially on the engineering technology students, will be presented.

I. Introduction

Microarrays are an orderly arrangement of DNA samples spotted onto glass slides or nylon membranes. Each spot is typically a sequence of DNA representing a distinct gene. To achieve the desired precision, these spots are typically applied by machines called microarrayers. The overall array dimensions are approximately 2 cm by 2 cm. Each spot of DNA is 100 to 200 microns in diameter and up to 20,000 can be applied to one slide [1]. Figure 1 shows how a microarray is printed.

The principle underlying the use of microarrays is DNA Hybridization. DNA is a double stranded molecule with each strand consisting of a stand of nucleotides. Each nucleotide consists of a sugar, phosphate, and nitrogenous base. There are four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and thymine (T). The A of one stand will only bind with the T of the other stand, and C will only bind with G. The stands of DNA can be separated or denatured. As seen in figure 2, Separate strands will bind only if their bases are complementary. Using these properties, an unknown
sequence of DNA can be identified by the binding of a labeled known sequence of DNA [3].

Figure 2. DNA hybridization.

Figure 3 illustrates a typical use of microarrays in a comparative gene expression study. In this experiment, one sample of cells is exposed to a treatment and the other is not. RNA is extracted from each cell sample and converted to cDNA. Each sample of cDNA is labeled with a different color fluorescent marker. The cDNA probes are allowed to hybridize with complementary DNA of the microarray. The spots are then visualized with laser excitation that makes the marker molecules fluoresce. A spot that is one color will indicate a specific gene expression of that sample. If both colors are present on a spot, then both samples expressed that gene. [3].

Figure 3. Comparative Hybridization Experiment.

1. Two cell samples are subjected to different treatments.
2. mRNA is extracted.
3. The mRNA from each sample is converted to cDNA and labeled with different color fluorescent molecules.
4. Fluorescent probes are hybridized to complementary DNA on the microarray.
5. Probes are visualized with laser excitation.
6. Completed microarray [3].

A fluorescent detection system is very effective, but requires a special fluorescent scanner to read the array that ranges in cost from $50,000 to $150,000. The student team has begun investigating an alternate detection scheme that uses a biotin label in place of the fluorescent molecule. As seen in Figure 4, a gold/antibody conjugate can be applied then followed by silver enhancement. The end result would be an array with varying intensities of monochromatic spots. While this system would only detect one sample at a time, a conventional scanner could be used to read the array. A high quality scanner such as this would cost less than $5,000. As mentioned above, microarrays can be used to study gene expression. This information can help determine metabolic pathways and responses to different treatments. This information can then be used to discover and detect abnormal gene expression associated with disease. Microarrays can also be used to detect mutations in alleles and associate these mutations with specific disorders. Such information can then be used as a diagnostic tool to screen for disease genes [5].
II. System Design

The main objective of the BioVenture Internship was to build and interface a DNA microarrayer. The microarrayer, designed by Stanford University Biochemistry Department, was built to the specifications outlined in the Mguide [8]. The machine was utilized to deposit, in a high-density format, nucleic acids onto solid substrates. Finally, utilizing a scanning device, these high-density slides would be digitized into an image format for DNA analysis. An understanding of the chemical and biological applications had to be learned along with the use of microarrays for hybridization and detection methodologies. The goal was to be accomplished by a student-team effort between the Engineering Technology and Biology Departments.

The Stanford microarrayer consisted of a basic system of three servomotor-powered linear rails mounted on a vibration isolation table that was controlled by a Galil DMC-1730 controller card mounted in a PC. Interfacing through an ICM-1900 breakout box, the control card communicates with three Compumotor TQ-10 amplifiers. These amplifiers supply torque-limiting current to drive the servomotors.

First, it will be helpful to have some understanding of what a servo system is. When talking about mechanical systems, typically some sort of input motion or signal is used to control the output motion of the system using some sort of feedback to maintain the output at a level selected by the input. The accelerator of a car is not a servo system because there is no feedback controlling the speed of the car. The operator provides that. A cruise control, however, is an example of a servo system. The cruise control measures the speed of the car, compares that to the desired velocity and adjusts the accelerator to keep the speed of the car constant. The servo system is actually an assembly of four parts: a servomotor, a gear reduction unit, a position-sensing device, and a control circuit. The DMC series from Galil is considered part of the servo systems. The control circuit, a controller board, is placed inside a host PC. Analog command signals and feedback signals are routed from all the drives and motors and bundled together to the controller board. A solitary processor is used to control many axes, which}
shares its processing power between all the axes under control. The following block diagram illustrates how these systems work:

![Block Diagram](image)

In the block diagram above there are two sections: the position sensing device and the velocity control circuit.

1. The Controller Card

The encoder from the servo motor works of a quadrature input signal. A quadrature encoder has two light receivers so that they can generate two different signals, both providing a difference in phase. There is a 90-degree phase difference, so that when the encoder is rotating forward, the generated signal is different than when reversing. This allows for direction detection by the sequence of phase variation. There are four different dual combinations that can be obtained from each period, thus the name quadrature encoder. The signal from the encoder is brought into the controller where a move profile is based upon step and direction signals from the indexer. For each step pulse received, the drive will make the motor turn one encoder count. Incoming step pulses represent commanded position and go into one of the inputs of a summing node. Incoming encoder counts represent actual position and go into the other input of the summing node. During a typical move, actual position will differ from commanded position. So actual position is subtracted from commanded position at the summing node and the result is the position error. This produced error is sent to the amplifiers as an analog voltage proportional to the error. The error signal is modified by a PID (Proportional, Integral, and Derivative) control loop then continues to the torque drive control board as a torque command. Controllers are designed to eliminate the need for continuous operator attention. Cruise control in a car and a house thermostat are common examples of how controllers are used to automatically adjust some variable to hold the measurement (or process variable) at the set point. The set point is where you would like the measurement to be. Error is defined as the difference between set point and measurement. \( \text{Error} = (\text{set-point}) - (\text{measurement}) \). The variable being adjusted is called the manipulated variable, which usually is equal to the output of the controller. The output of PID controllers will change in response to a change in measurement or set point. A proportional controller will have the effect of reducing the rise time and will reduce, but never eliminate, the steady-state error. An integral controller will have the effect of eliminating the steady-state error, but it may make the transient response worse. A derivative control will have the effect of increasing the stability of the system, reducing the overshoot, and improving the transient response. With a proportional controller, offset (deviation from set-point) is present. With integral action, the controller output is proportional to the amount of time the error is present. Integral action eliminates offset. With derivative action, the controller output is proportional to...
the rate of change of the measurement or error. The controller output is calculated by the rate of change of the measurement with time. Derivative action can compensate for a changing measurement. Thus derivative takes action to inhibit more rapid changes of the measurement than proportional action. Derivative is often used to avoid overshoot. Derivative action can stabilize loops since it adds phase lead. Generally, if you use derivative action, more controller gain and reset can be used.

2. The Velocity Control Circuit

This is located in the TQ-10 amplifier as the torque drive circuit. The amplifiers receive a position velocity compensation signal from the controller card. This analog voltage is a torque command that represents commanded current. Inside the TQ-10 the torque command goes into one of the inputs of a summing node. A feedback signal representing actual motor current, which is derived from the trapezoidal commutation of the Hall Effect sensors, goes into the other input. When actual current is subtracted form commanded current at the summing node, the difference is current error. The resulting current error signal goes through an error amplifier whose output controls a pulse width modulation (PWM) circuit. If actual current is too low, PWM circuit will send longer pulses to the drive’s power stage. If actual current is too high, the PWM circuit sends shorter pulses, resulting in less motor current. Many modern speed controllers for DC motors use this technique of Pulse Width Modulation (PWM) to control the speed of a motor. By varying the duty cycle (width) of this switched voltage, the effective average voltage can be lowered (narrow width pulse) or raised (wide pulse). This produces the effect of a linear change in current.

The servomotor is an electrically driven machine. The operating principle of a servomotor is similar to DC motors. There are two significant parts of the DC motor, the rotor and the commutator. The rotor contains several coils, which are often referred to as the “armature”. To connect the coils together, a device called a commutator is used. The commutator distributes current to the appropriate coils. Two stationary conducting brushes provide current to the commutator. As the rotor and attached commutator spin, the brushes come in contact with different areas on the commutator, which cause different coils in the rotor to conduct. This causes an armature flux to be created. The commutator ensures that the armature flux is always aligned in a constant direction as the rotor spins. So no matter what position the rotor is in, the armature flux will always be aligned, as shown here. If the armature current is reversed, then the direction of the armature flux will be reversed.
A servomotor has an output shaft. This shaft can be positioned to specific angular positions by sending the servomotor a coded signal. As long as the signal exists on the input line the servo will maintain the angular position of the shaft. As the coded signal changes, the angular position of the shaft changes also. Therefore, a servomotor, in this sense, is simply a mechanism that slaves some sort of action to some sort of input. The function of the servomotor is to receive a control signal that represents a desired output position of the servo shaft, and apply power to its DC motor until its shaft turns to that position. It uses a position-sensing device to determine the rotational position of the shaft, so it knows which way the motor must turn to move the shaft to the commanded position.

III. Accomplishments

At the beginning of this project, the student team had to do a literature survey in order to understand basic principles in DNA analysis using microarrays and to see what others have done in the field. In addition, the engineering technology students had to review and understand basic biological and chemistry concepts while biology students had to learn basic electronics and control concepts. The student team worked together and helped each other learn the “foreign” concepts. While doing the literature review, students started to order the microarrayer parts from
various suppliers. The students worked closely with BioVentures engineers and biologists to get the most suitable parts for the project. The students then assembled and tested each part individually. When all parts were ready, the microarrayer was assembled and then tested. Various adjustments were made and many unexpected problems were resolved. At the end, it was really great to see the students while watching the microarrayer creating arrays on slides. Although students have spent long hours and much more time than they expected to spend on this project, however, they felt that the experience gained outweighed the long hours.

IV. Conclusions

A multidisciplinary student teams from the engineering technology and biology departments at Middle Tennessee State University has built and tested a microarrayer, which was used to deposit arrays of samples on slides. Several problems were overcome, and team cooperation was realized to be an important ingredient to the completion of a project. One of the students wrote is his report: “As a graduate student in the Engineering Tech. Department with a concentration in Computer Engineering Technology, this project reinforced the knowledge gained from institutionalized learning with valuable work environment experience in robotic design principles. Also, this construction project furnished supplementary knowledge and experience in microarrayer technology.”

IV. References


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Dr. Saleh M. Sbenaty is currently a Professor of Engineering Technology at Middle Tennessee State University. He received the BS degree in Electrical Engineering from Damascus University, Syria and the MS and Ph.D. degrees in EE from Tennessee Tech. University. He is actively engaged in curriculum development for engineering and technological education. He has written and co-authored several case studies. He is also conducting research in the area of mass spectrometry, power electronics, instrumentation, and lasers.