MICROARRAYER AND LUMINESCENCE MEASUREMENT

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ABSTRACT

The function of Microarrayer (also called Array Spotter) [1] [2] [5] is used in the field of biomedical research to place a matrix of tiny spots of pre-prepared substances onto a solid substrate (such as treated glass). The spots are then excited by suitable light to emit luminescence which is monitored with a fluorescence microscope, or the like, to determine related information. For example, the information content of the genome is carried hereditarily as DNA, the size composition of a given genomic sequence determines the form and function of the resultant organism. Our project is to design and build automatic arrayer (usually called a robot in the micro-robotics) and the measurement system. This project is more of experimenting and training than to develop a real industry – standard equipment which requires expensive imported items.

1. MICROARRAYER

The problem is to place an matrix (array) of spots of substance liquid on a surface – treated glass substrate (Figure 1). The liquid is contained in corresponding array of wells (Figure 2). A corresponding array of pins takes a every small amount of liquid into each pin and transfer it onto the glass substrate in the form of tiny spots. This short description on shows that there are many things to solve in order to obtain necessary quality (equal spot spacing and size, same amount of liquid . . .).

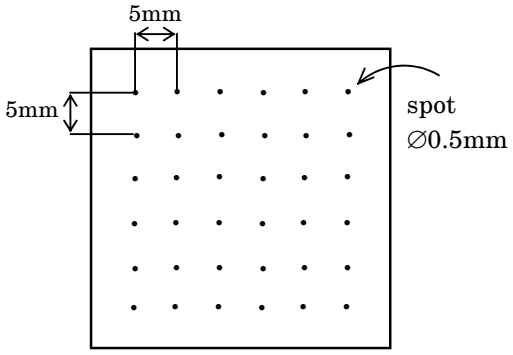

Fig. 1 Glass base and 6x6-spot array

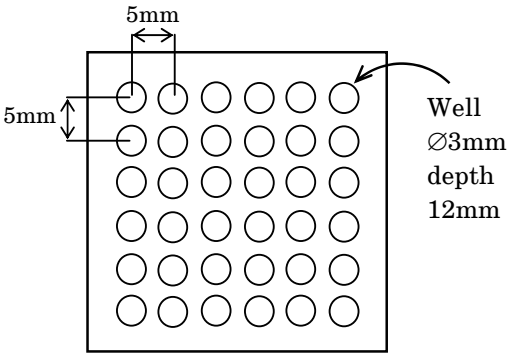

Fig. 2 Thick mica plate with 6x6-well array

We were asked to build a manual equipment and an automatic one. Only through laborious experimenting we could come out with a manually operate equipment as shown in Figure 3. The top part with a handle and with a tip assembly (containing an array of 6x6 pins) is moved to the left and pressed down in order the pin tips will dip into the corresponding wells to take a small amount of liquid each. The top part is then moved to right and pressed down to transfer the liquid into spots on the glass base. There are several adjustable items (springs, adjustable thumbwheels, limit obstacles . . .) so that the equipment can work properly.

Fig.3 The manual microarrayer

Experiences from the manual arrayer allowed us to realize an automatic robot operated by compressed air and controlled by a microcontroller (Figure 4). It is much bigger than necessary (hence very sturdy and impressive) just because we couldn't get smaller compressed air operated linear positioning axes. Notice the triple – tube

Fig. 4 The automatic arrayer (robot)

compressed air operated linear axis on the top to move the printing head (pin matrix) forth and back to transfer liquid on the right (not seen) to make spots on glass base on the left (not seen). Especially, our robot can operate in both manual and automatic models.

2. LUMINESCENCE MEASUREMENT

A laser shines strong excitation light onto each substance spot which emits an extremely weak luminescence. Our duty is to measure the intensity of this radiation. Real measurement is highly sophisticated and expensive. For industry – standard equipment one needs a precision optical system with beam splitter and a photomultiplier, or a scanning luminescence microscope, or turns to imaging method [5]. Here, mainly for experimenting, we develop our own system, avoiding using expensive items.

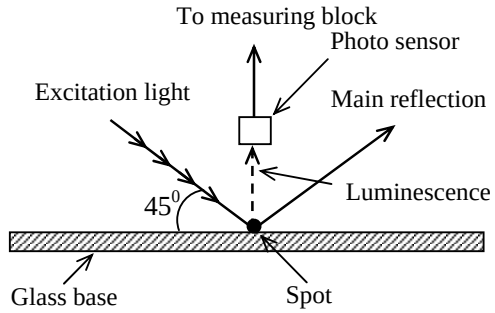

Fig. 5 arrangement for excitation light and photo sensor

First we arrange the measurement as in Figure 5. The laser light is incident onto the spot at an angle of 45^o so as not to reflect onto the photo sensor which is placed quite close to the spot. The excitation laser wavelength should be as close as possible to the most sensible wavelength of the substance. The trouble is that the wavelength of the luminescence is very close to the excitation wavelength. For example, the two wavelengths in the case of CY3 are 565nm and 550nm respectively. Besides, the luminescence intensity is extremely weak and in the co-existence with many sources of noise. We used a green laser at 532nm which is rather close to the excitation wavelength of 550nm of CY3 (no such laser light of 550nm wavelength was available).
The current generated by the photo sensor is converted to voltage, then filtered, amplified, converted to digital and input to computer via the serial port. To suppress noise as much as possible, high quality differential amplifier must be used. So why we see a matched pair of photo sensors in the front of Figure 6, one collects luminescence as well as a certain amount of laser light from the excited substance, the other receives only the laser light. Especially, we position the two sensors so that they receive about the same amount of

laser light and other extraneous noises and light interference.

Figure 6 is the block diagram of electronic system comprising two processing blocks. The strategic component in the analog part is the Instrumentation Amplifier INA114 from

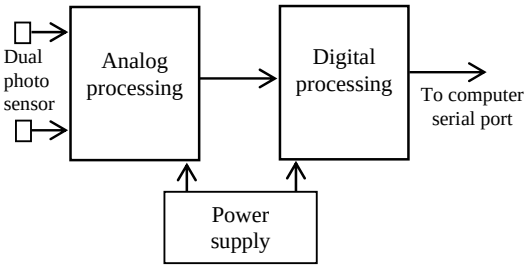

Fig.6 Block diagram of the electronic processing

BURR-BROWN. The filter is a 4th order low-pass Butterworth with cutoff frequency at 1Hz. In the digital part, apart from A/D converter and associated circuits we also add microcontroller AT89C51 and RS232 converter MAX232 for interfacing with computer serial port. Due to the limitation of space the circuit diagrams are not included here.

Fig. 7 Luminescence measurement system

Figure 7 is the photo of the measuring system. The box on the right is an intricate manually operated mechanism to move sequentially the substance spots (an array of 6×6 spots have been created as described in section 1) to get underneath the fixed stationary laser light – photo sensor combination. The X-Y movement is quite easy and smooth. Of course this mechanism can be made automatic but the manual way also has some advantages (actually we were asked to make a manual device). On the box front we can see the Offset button (to adjust the offset of the Instrumentation Amplifier) and the Calibrate button (for calibration). These two functions can be done automatically from the computer but this way is rather complicated and might not be as reliable.

The box on the left (in front of the monitor) contains all electronic circuitry and power supply, except the pre amplifier which is in the right box.

3. SOFTWARE

The software of the luminescence measurement consists of two programs:

- Monitor program loaded into flash ROM of the microcontroller.
- Program for data acquisition and processing of the computer.

Due to space limitation, program flow graphs are not included here. Figure 8 is the program interface, figure 9 shows an example of measurement, and the last figure 10 is an example of graphical plot of measured data.

Fig.8 Program interface

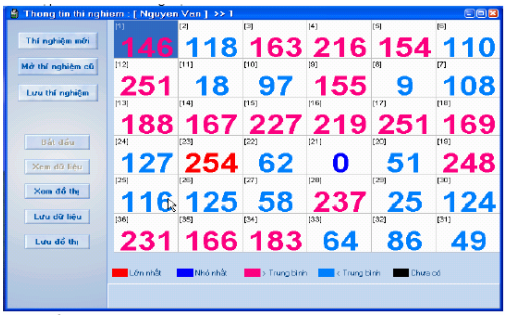

Fig.9 Example of experimental result

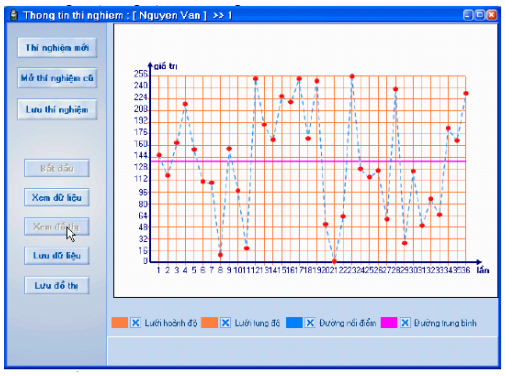

Fig.10 Graphical plot

4. CONCLUSION

The project consists of two parts: Microarrayer and Luminescence measurement. For the arrayer we spent much time in designing and building a manually operated device, and an automatic robot operated by compressed air and controlled by a microcontroller. For the luminescence measurement we went the almost purely electronic way, instead of using optical or imaging method, because it suits us in term of cost. For the luminescence measurement we also developed an effective manual mechanism to move the spots step-by-step. In summary, both the mechanics and the electronics of the project have been completed with a good result, judging on the chosen direction and means. Although the whole system is not precise enough for scientific laboratory use but can serve very well experimenting and training purpose.

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