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# Modification of A Commercial Inkjet Printer for Protein Spotting

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**Abstract.** Dispensing a minute amount of reagent is important in many applications, especially in immunoassays, which the reagents are often expensive. Unfortunately, most of commercial robotic systems are costly and complex. In this study, we described a process to modify a commercial inkjet printer for protein spotting onto a nitrocellulose membrane glass slide. The custom-made printing system was composed of 2 inkjet printers. The first printer was used to print the protein solution, while the other was used to send out a paper signal. Bovine serum albumin (BSA) was selected as a model protein in this study. The resolution of the printing system was determined and its reproducibility was evaluated. Our result has shown that the concentration of the protein solution did not affect the performance of the printing system. In addition, the coefficients of variability from the intra- and inter-assays indicate that the protein printing results were reproducible.

**Keywords:** Inkjet printer, Protein printing, Protein spotting, Printer modification

## 1. Introduction

An immunoassay has been employed in many clinical tests, for example the determination of biomarkers for diagnosis of life threatening diseases, because of its high sensitivity and specificity. This method detects and quantifies specific substances, which often are proteins, via an immunological reaction between antibody and antigen. This assay is generally expensive due to the high cost of antibody. To overcome this problem, a microscale immunoassay has been developed to reduce the reagent consumption issue, as well as to increase the binding rate between antibody and antigen due to high surface area to volume ratio [1]. In this microscale system, a minute amount of antibody is deposited on to a pretreated glass slide. Unfortunately, manually spotting nanoliter volume of antibody solution is difficult to control, resulting in variation in the amount of immobilized antibody and spot sizes. Commercial robotic systems have been suggested. Although these systems provide superior performance, they require well-trained personnel and large investment.

Until recently, several researchers have investigated the use of inkjet printing as a low cost alternative to allow precise deposition of small volume of biological fluids on substrates [2]. An inkjet printer is capable of generating spots as small as 50 μm with a resolution of 80 μm [3]. The previous study has shown that the functionality and activity of the biomolecules remained the same after being dispensed using a thermal inkjet printer [4], thus proving the potential use of thermal inkjet technology for the dispensing of biological fluids. Several approaches have been used to modify the hardware and software of commercial inkjet printers. The difficulty in modifying the printer hardware depends entirely on the printers' brands and models. The modification of the printer software can be tricky. It can only be done if researchers get a copy of the codes from the manufacturers.

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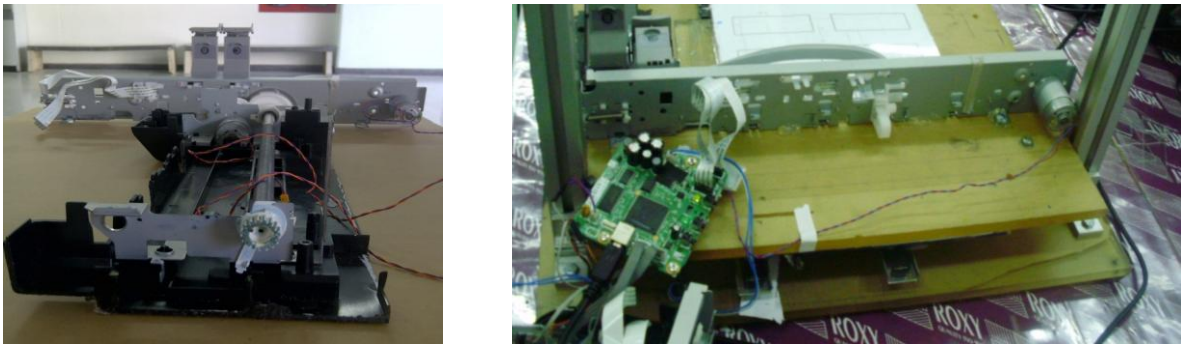
In this study, we described simple steps to modify Cannon IP 2700 inkjet printers to print spots of protein solution onto a nitrocellulose membrane glass slide. We only made some changes to the hardware of the thermal printer without any alteration to the printer software. A mixture of bovine serum albumin (BSA) and a red dye was used as a model protein solution in this study. The colour dye was added for a visualization purpose only. The performance of the custom-made printing system and the print resolution were evaluated.

## 2. Materials and Methods

### 2.1. Modification of the Inkjet Printer

Two Cannon IP 2007 inkjet printers were used in this study. For the first inkjet printer, its paper feeding mechanisms such as a tray, guide-rails, rolls, and optical sensors, were removed, leaving only the control board and the print head visible (Fig. 1a). The remaining components were attached to two 48 cm × 60 cm × 1 cm (W × L × D) acrylic boards. The print head was assembled onto the top board, while the bottom board which was glued to stainless steel guide-rails was used as a slide holder and a slide feeder (Fig. 1b).



(a) (b)

Fig. 1: The first inkjet printer after (a) the removal of several components, and (b) being assembled onto two acrylic boards.

For the second printer, the plastic cover was completely removed showing only the working parts of the printer. The optical sensors of the second printer were wired to the control board of the first printer. With this arrangement, the second printer sensed the incoming signal and sent a command to the first printer to start printing. A schematic representation of the printing system is shown in Fig. 2.

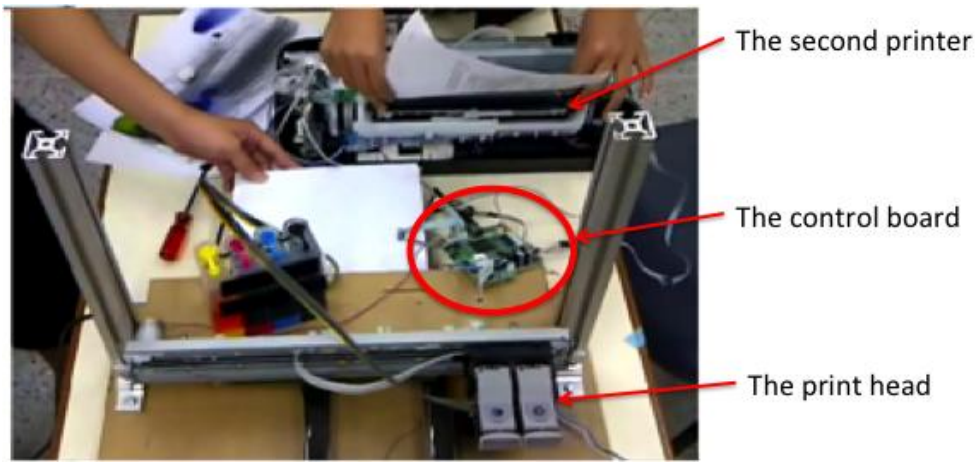



Fig. 2: A schematic representation of a custom inkjet printing system

The modification of the ink cartridge was also necessary for a low volume dispensing application. The original sponge inside the black ink cartridge was removed and the cartridge was washed repeatedly with 95% ethanol until all ink residuals disappeared. To reduce the printing medium consumption, a 1 cm × 1 cm × 1 cm (W × L × D) polystyrene block was placed over the printer nozzle using silicone adhesive (Fig. 3) and stuffed with a clean sponge.

Fig. 3: The modified ink cartridge

## 2.2. Printing Procedure and Performance

A mixture of bovine serum albumin (BSA) and a red colour dye in deionized water was used as a printing medium for this study. One hundred micro litres of the printing medium was loaded into the previously modified black ink cartridge. Print patterns drawn using Microsoft Word comprise solid circles with various diameters ranging from 200 μm to 5 mm to determine the resolution of this printing system. In addition, the effect of the protein concentration on the print resolution was determined by varying BSA concentrations. The size and colour intensity of each printed spot was analysed using Scion image analysis software.

The reproducibility and repeatability of the printing system were assessed using intra- and inter-assays, respectively, in terms of coefficient of variability which was calculated as shown in the equation below.

$$\%CV = \frac{S\,\text{tan dard deviation}}{M\text{ean}} \times 100$$

The BSA solution was repeatedly printed as a 1 mm spot 20 times on one nitrocellulose membrane slide to test the intra-assay precision. Twenty replicates of 1 mm spots on 5 separate slides were used to determine the inter-assay precision.

# 3. Results and Discussion

## 3.1. Modification of the Inkjet Printer

Two Cannon IP 2007 inkjet printers used in this study employ thermal technology to deliver small droplets of liquid solution onto a desired substrate. The liquid is heated to form vapour bubbles inside a series of microchamber. Expansion of vapour bubbles due to heat causes the liquid to be expelled from the nozzle of 10 μm in diameter [5]. Newer model printers contain numerous checks by optical sensors to prevent the printers from performing tasks beyond their specification, adding complication to the modification process. Several approaches have been used. For example, a new code was written to override the paper mechanism of the printer [5]. In another study, all of the optical sensors were removed and a signal was produced by a MATLAB routine [6]. These two methods have proven to be difficult unless the original code is available or researchers have thorough understanding of signal processing. In this study, we have chosen a simpler way to print our protein solution using one printer by feeding paper through the second printer to bypass the paper checks. Most of the printer hardware parts were not modified to prevent any possible malfunction of the printing system. In this arrangement, the print head moved horizontally printing protein solution onto desired substrates, while the acrylic board moved vertically feeding the substrates into the printing system.

Another major constraint in building this custom printing system is the ability to function with low volume reagent. Often times, the reagents used in these types of applications are protein, antibody and DNA which are expensive and only available in small amounts. Therefore, the printer's ink cartridge which can hold as much as 10 ml needed to be modified to accommodate small volume of samples. This part was achieved by adding a small polystyrene compartment over a nozzle inside the cartridge and stuffed with a much smaller polyurethane sponge (Fig. 3). The sponge is important in providing constant pressure to the nozzle regardless of the hydrostatic level, preventing leakage [6]. After the modification, a sample with volume as low as 100 μl can be used with this printing system.

## 3.2. Performance of the Printing System

A substrate selected in this study is a nitrocellulose membrane slide. Nitrocellulose membrane is commonly used in many biological applications such as Western Blotting, protein microarray and immunoassays. The membrane can bind to protein with high capacity via both hydrophobic interaction and electrostatic interaction [7]. It can also preserve the tertiary structure of the proteins. As a result, our printing medium containing BSA would bind to the substrate immediately after being printed. An addition of a red dye was for visualization only. It was previously tested to show that the addition of the dye has no effect on the solution viscosity (data not shown.)

### 3.2.1. Limitation of the Printing System

Solid circles with diameters ranging from 200 μm to 5 mm were printed on nitrocellulose membrane slides to determine the resolution of the printing system. Any spots with a diameter below 450 μm were not visible on the slide. The printing system was able to print protein spots with diameters between 450 μm to 900 μm (Fig. 4a). However, these spots appeared to be irregular which would possibly cause variability in experiments. The protein spots with diameters from 1 mm to 5 mm showed circular shape similar to the design in Microsoft Word program (Fig. 4b). Our result indicates that the smallest spot that this printing system can print is 1 mm which is sufficient for an application in microscale immunoassays. However, this system should be not be used in a microarray application in which spots of proteins or DNA are required to be significantly smaller.

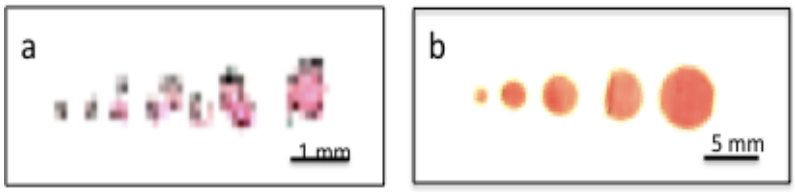



Fig. 4: Images of the protein spots on a nitrocellulose membrane slide with diameters between (a) 450 μm to 900 μm and (b) 1 to 5 mm. The scale bars represent 1 mm in (a) and 5 mm in (b).

### 3.2.2. Effect of Protein Concentrations on the Print Quality

It is possible that an increase in the concentration of BSA in the mixture might have increased the viscosity of the printing medium, causing a problem in droplet ejection and eventually clogging the nozzle. To evaluate the effect of the protein concentration on the printing performance, various concentrations of BSA (0, 1, 2, and 3 %w/v) in the printing medium were printed onto the membrane slides. These concentrations are normally used in various immunoassay applications. The diameters and colour intensities of the printed spots were then analysed, as shown in Fig. 5. It is clear that the protein concentration had no effect on the print results as the diameter and colour intensity of each spot were similar.

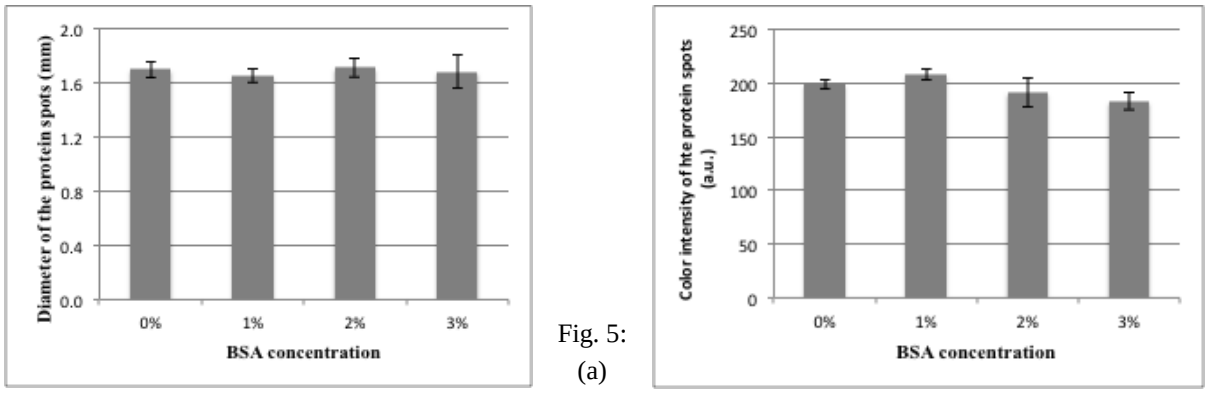



Fig. 5: (a) The average diameters and (b) colour intensities of the protein spots printed using the printing medium containing various concentrations of BSA.

### 3.2.3. Repeatability and Reproducibility of the Printing System

The intra-assay is used to determine the spot-to-spot variability, while the inter-assay shows the slide-to-slide reproducibility. As shown in Table 1, the intra and inter-assay CVs of the diameter and the colour intensity were well below 5%. According to the guideline for biological research studies, the intra- and inter-assay CVs should be less than 10% and 15%, respectively [8]. Thus, based on the CVs reported in this study, the performance of our printing system is considered reliable.

Table 1: Intra- and Inter assay CVs of the protein spots printed using our custom-made printing system

| | Intra-assay CV (%) | Inter-assay CV (%) |
|---|---|---|
| Spot size | 2.65 | 4.29 |
| Colour intensity | 0.76 | 2.02 |

## 4. Acknowledgement

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