Design and development of manufacturable protein arrays

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Abstract

The development and optimization of manufacturable protein arrays requires researchers to address numerous technical challenges. The elements that contribute to the appropriate performance of arrays from a manufacturing perspective include the design of the array itself, the type of dispensing system used, the environment in which dispensing is carried out and appropriate choices of substrates, reagents and buffers. Careful consideration must also be given to process design and process parameters such as cleaning of the system and degassing and filtering of reagents. As a result, optimizing the process parameters to manufacture high quality protein arrays can be time consuming and costly.

As a manufacturer of nanoliter dispensing equipment, BioDot has had extensive experience with the development of manufacturing processes for the protein array industry. This poster highlights some of the main issues relating to appropriate process design for manufacturable protein arrays, and provides data demonstrating solutions to the issues addressed, including data on the contribution of appropriate platform design, substrate choice, buffer choices, arrayer cleaning, system degassing, and environmental control, key factors to the manufacturer's ability to robustly produce protein microarrays.

1. Array Design and Platform Scalability

Introduction

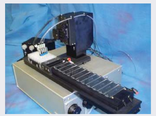
Commercially available nano- and pico-liter dispenser platforms typically are required to perform a wide variety of applications at a variety of scales, from laboratory to full scale production. Platform and dispenser design must exhibit some degree of flexibility to overcome these challenges.

In highly demanding arraying applications, dispensing platforms typically should include a number of features critical to the process. These include aspirate and dispense capability, wash and dry stations for dispense tips carefully designed to avoid carry-over issues, humidity control, and the ability to customize deck configurations for different substrates.

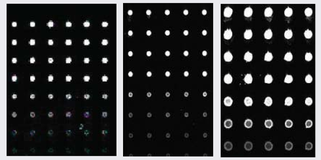
The dispensing technology used is obviously critical to the success of the arraying process. BioDot's BioJet Plus technology is designed to provide a scalable solution to low nanoliter to microliter dispensing issues. The BioJet Plus technology utilizes the synchronization between the action of a high precision pump, microsolenoid valve and robotic motion to create highly accurate and repeatable dispenses in the nanoliter range that can be placed on a substrate with extremely high positional accuracy at high speed. This dispensing technology is adaptable to a variety of dispensing platforms and is completely scalable from a single channel on a small R&D platform to a multi-channel overhead gantry production system without significant revalidation of the dispensing process.

Careful process design, taking equipment design limitations into account, is critical to ensuring a smooth transition from research protein microarrays into a scaled up production line.

Flexibility of the design: BioDot AD5000 manufacturing printer, AD3200 research development printer and AD1500 research printer. BioDot arrayers are designed to accommodate different dispensing platforms for slides, microtiter plates, biochips, membranes, sheets of biosensors, etc…



Scalability of dispensing channels for faster throughput: BioDot's arrayer equipped with one dispensing channel can print twenty microarrays in 6 min 14 seconds, each array being 8 x 12 dots. It would take 3 min 7 seconds to do the same sporting experiment with two dispensing channels (14 nL spots, 1.02 mm center-to-center distance, 18 mm spacing between arrays).



Dot volume	20 nL	100 nL	500 nL
spot size:	500 µm	800 µm	1400 µm

Experiment: Methods and Results

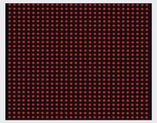
Necessity to use different sizes of dispensing channel to obtain good protein microarrays over the 20 – 500 nL range of spot volumes. BioDot printer can accommodate different size of dispensing tips (the most common being 100 micron and 190 micron orifices). Larger spot size will suffer in their morphology if the size of the dispensing channel is not appropriate.

Arraying performed on BioDot AD5000 equipped with one dispensing channel and a humidity chamber. Eight different concentrations of pRaglgg were spotted under 50% humidity onto amine reactive OptArray slides. Print Buffer: 0.2M NaPO4 pH8.5, 0.005% CHAPS, 0.5% glycerol, and 0.01% BSA. Protein concentration doubled from row to row (bottom was 1.6 ng/mL top is 200 ng/mL). All slides were dried for two hours in a 50% humidity chamber. The slides were blocked for 1 hour in 50 mM NHEtOH and 1x PBS, then rinsed, then incubated with glgg-Cy5 at 10 ng/mL in 1x PBS for 1 hour. The images were obtained on a Tecan LS400 scanner, at 633/670 nm, PMT 125, 10 microns.

Volume delivered (nL)	Average CV% over 960 drops	Stdev over 960 drops
20	7.10	3.8
30	7.52	4.0
100	5.00	2.6
500	1.41	0.6

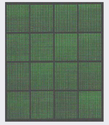
Table 1: Coefficient of Variation (%CV) measured by fluorescence detection method on BioDot AD5000 equipped with 8 dispensing channel. Each %CV measures the variability in the volume delivered over 960 drops (except noted otherwise). Each dispensing channel delivers 120 drops,* over 840 drops only

For higher density microarrays, a non-contact piezo-electric arrayer will deliver high throughput and high quality prints.



Robust technology for picoliter non contact dispensing: Arraying performed on BioDot SciFlex, 300 pL drop, 400 m center-to-center pitch. CVs are estimated to be around 1%. SciFlex robust technology is based on fluid pressure, piezo-electric impulse, and glass capillary.

By contrast, pin arrayers operate in contact mode and are thus more prone to clogging



Contact-dispensing: Microarrays printed at PST1 using 75X75µm silicon pins spaced on 4.5mm centers with a spot spacing of 170µm (above). The above image shows all the spots (including prespotting) printed from a single uptake volume of 100nL. Arrays printed with a spot spacing of 145µm (below) using 75x75µm tips.

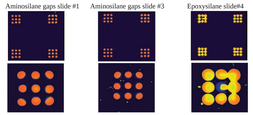
	Tip Size 100 x 100µm	Tip Size 75 x 75µm	Tip Size 50 x 50µm
Average spot diameter	100-130 µm	75-110µm	50-80µm
% CV	7%	5%	4%
Total number of spots per one dip	350-400	400-500	500-600
Volumetric uptake	~ 0.1µL	~0.1µL	~0.1µL

Table 2 Statistical analysis of arrays printed with Si Pins (Arrays are printed on one slide in test print mode)

2. Substrate Choice

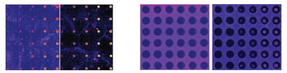
Introduction

The substrate on which the protein microarrays will be printed is critical when evaluating a robotic arrayer for protein microarrays. Different glass slides carry different surface chemistries. These distinct surface chemistries are responsible for different interactions with the protein microarray: the spots may spread more on one surface than on another, causing a weaker signal, possible coalescence of the spots, and splattering. Identification of the best possible substrate for a specific protein arraying process is critical to success.



Experiment: Methods and Results

Illustration of different spot sizes and qualities in a protein microarray spotted on two different types of slides: epoxysilane coated glass slides and aminosilane gaps slides. Arraying performed on BioDot AD3200 unit equipped with one dispensing channel and an in-line degasser. Each slide was spotted with fourteen microarrays containing 9 dots of 20 nL each of protein solutions (125 ug/mL of goat anti-mouse in 0.05 M carbonate coupling buffer). Upon developing, the slides were scanned on an Axon 4200AL scanner using laser settings of 40 and PMT of 230.

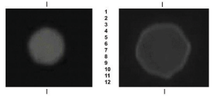


Fast Slide

Teflon poly-L-lysine coated slides

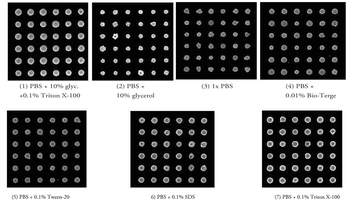
Spot size differences between antibody arrays on S&S Fast Slides and on Teflon poly-L-lysine coated slides. Arraying performed on BioDot AD5000 arrayer equipped with one dispensing channel. Each slide was spotted with 6x6 arrays of 12 nL dots of mouse HCG at 6 different concentrations in PBS buffer. Upon printing, the slides were dried, blocked using 1% FCS in PBS, washed, incubated with 1 ug/mL of goat anti-mouse IgG conjugated with AlexaFluor488, washed, dried and scanned on an IsoCyte™ laser (488 nm). For optimum picture quality, mineral oil was used to change the refractive index of the membrane and to enhance signal prior to scanning. Dot size was estimated to be between 400 and 550 microns on Fast Slides, and between 700 and 900 microns on Teflon coated poly-L-lysine slides. The protein concentration was 0, 0.12, 0.6, 1.2, 6 and 12 ng from left to right on the array.

3. Arraying Buffer Choice



Dependency of spot size on substrate. IgG in PBS on ζ-Grip™, (Miriggene) and on Fast Slides (Schleicher and Schuell). Smaller spots on ζ-Grip™ had a higher intensity, which allowed them to be read by a simple colorimetric method, instead of a fluorescence detection method.

Several arraying buffers are commercially available for protein microarrays, but the user must keep in mind that the manufacturer usually optimizes the buffer for one type of glass substrate. Protein array wash buffer and protein array blocking buffer are also critical to the process. The latter is usually developed for one specific detection method. Protein array buffers are typically designed to enhance protein stability and signal intensity. However, the importance of arraying buffers on the spot roundness and size for protein microarrays is high: depending on the arraying buffer, in our hands spot diameter has been seen to vary by up to 200 um, about 28% of the total diameter of the spot (see table 1). For all these reasons, the user would greatly benefit from developing his own optimized buffer for his specific protein microarray. BioDot offers simple programs to achieve this iterative process efficiently using robotic nanoliter dispensing equipment.



Experiment: Methods and Results. Evaluation of seven spotting solutions. The spot roundness and size for each arraying buffer was evaluated at 10 nL drop volumes. All solutions contained the same concentration of antibodies (0.125 mg/mL of donkey anti-goat antibody) but had different salts, detergents, sugars and other buffers additives. The arrays were generated on BioDot AD5000 with one BioJetPlus dispensing channel equipped with a helium degasser. The dispensing height was 0.14 mm from the surface of the slide. Upon printing, the slides were dried in desiccating chambers, shipped, and then blocked with 1 x S&S Protein Array Blocking Buffer, incubated with a biotinlated antibody derived from goat, washed, incubated with streptavidin-Cy5 conjugate, washed and dried. The slides were then scanned with P.E. Scan Array4000 imager. The buffer composed of PBS, 10% glycerol, and 0.1% Triton X - 100 yields spotted with the best combination of roundness, spot size consistency, and morphology. This buffer had the highest spot intensity, the best aspect and heterogeneity measure, and was near the top in both roundness and margination. However, it also yields the largest spots.

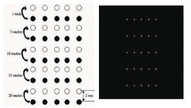
Buffer #	Specific intensity	CV intensity	Ave Diameter (um)	STDEV	CV
(1)	40065	8	777	47	6.1%
(2)	35794	7	555	54	9.7%
(3)	26003	9	604	43	7.1%
(4)	26460	4	644	69	10.8%
(5)	23608	5	648	34	5.2%
(6)	32597	10	716	35	4.8%
(7)	37659	10	740	35	4.8%

Table 1: average spot diameter of protein in various print buffers

4. Arrayer Cleaning

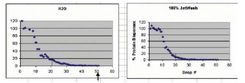
Introduction

Mechanical cleaning of proteins from dispensing tips can be achieved using a simple aspirate-and-dispense wash strategy. However, when defining a cleaning strategy, the customer should consider the particular characteristics of their proteins and choose cleaning reagents appropriately. Certain proteins or protein diluents may be incompatible with water and prefer organic based cleaning solutions. Customers should validate their own cleaning procedures for this reason. In high throughput non-contact arraying, dispensing micro-droplets (nL to uL) of concentrated aqueous solutions of protein can cause blockage of ceramic tips with subsequent damage. Accuracy, precision and also production costs and rate can be compromised in both contact and non-contact arraying when concentrated viscous reagents containing protein, nucleic acid, binders and other polymers are used. In the contact approach, production time allotted to pin cleaning can exceed the arraying period. Non-contact printing generally suffers fewer problems with reagent clotting because liquid is exposed to the evaporative effects of air for much briefer intervals. However, when highly concentrated solutions are used and hardware washing is infrequent, tip blockage with subsequent over-pressurization induces print failures.



Experiment: Methods and Results

BioDot has developed and validated the efficiency of its own mechanical in-process cleaning procedures to prevent carryover of protein between aspirated samples. In the array shown above, a full circle represents a 20 nL drop of antibody IG-AP, an empty circle represent a 20 nL drop of buffer. Only spots in lines containing the original IgG-AP reagent were visible upon development of the slides. No spots developed in any of the lines containing wash solution after washing of the tips. Therefore, no evidence of carryover of the IgG-AP was seen on any slide under any wash condition, even after only a single wash cycle. IgG was used as a representative "sticky" protein. The alkaline phosphatase method of development on Zeta Grip chips was chosen because of previously demonstrated sensitivity and ease of use. The results indicate that the BioJet Plus ceramic tips do not retain substantial amounts of protein and simple water rinsing of the tips is sufficient to remove residual IgG after dispensing.



Efficient washing steps: Elimination of protein by various washing agents when compared to water. Water by itself is capable of washing protein from the system. Study performed on BioDot AD5000.

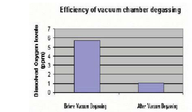
5. System Degassing

Introduction

Non-contact dispensing technology exhibits high level of reliability for small volume dispensing under the proper experimental conditions. The accuracy and the precision of the drop volume are ensured by the steady-state pressure (SSP) inside the dispensing channel. This pressure will only be steady if the column of fluid inside the dispensing channel is continuous –meaning in the absence of any air bubbles. De-aeration of the backing fluid is thus critical to maintain the fluid path of non-contact dispensers free of any air bubble. The presence of any air bubble would lead to inaccurate dispensed volumes.

Flow-through vacuum degassing chamber with a single amorphous perfluorinated copolymer (Teflon® AF) degassing membrane. This compact design comprises continuously vented mini vacuum pump with a unitary PTFE diaphragm. Degassing efficiency is equal to fifty (50) times the degassing efficiency of other PTFE designs. Several vacuum degassing modules may be mounted in parallel on one arrayer. Eliminate any set-up time required for degassing prior to any dispensing experiment.

Experiment: Methods and Results



Efficiency of flow-through vacuum degassing chamber to lower the level of dissolved gas inside a fluid. Experiment performed with BioDot syringe pump. The fill flow rate is 30 uL /sec. The prime flow rate is 60 uL/sec. The vacuum efficiency is constant overtime and guarantees the degassing efficiency.

6. Environmental Control

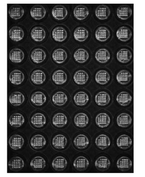
Introduction

During and after arraying, it is crucial to maintain a controlled environment. Clean rooms are usually the best environment to obtain high quality protein microarrays. The presence of dust during the arraying process may cause splattering of the spots due to particulates on the substrate surface or clogging of the dispensing nozzle. Once a protein array has been spotted successfully, the drying process deserves particular attention. If this process is not thoroughly thought through, the protein array may turn out useless, as the formation of doughnuts instead of homogeneous spots will affect the interpretation of any quantization software. In most cases, it is necessary to maintain a relatively high humidity atmosphere (up to 60-70%) within the print enclosure to prevent the spots from drying out from their external side. The drying out of spots is deleterious in two ways: it creates a dry protein ridge called a "doughnut effect", which affects signal intensity measurements, and it may deactivate the proteins and thus produce false negatives. The addition of glycerol inside a protein solution may be useful to avoid the "doughnut effect" but it creates several challenges. Most commercially available arrayers have difficulties spotting solutions with high glycerol content. BioDot is capable of arraying high quality protein microarrays containing up to 10 % glycerol using BioJet Plus technology. However, the drying time is considerably increased by the addition of glycerol. Unless the arraying buffer possesses glycerol, BioDot recommends leaving the slides spotted with a protein array inside a humid environment for at least 1 hour. This allows the protein array to dry slowly in a controlled manner, which usually works well to avoid the "doughnut effect".

Experiment: Methods and Results

Arraying performed on BioDot AD3200 equipped with one dispensing channel and flow-through vacuum degasser. The addition of 5-9% glycerol to their solutions helped avoiding drying out the 20 nL protein spot but the key to this issue resided mainly in the controlled drying environment. For this experiment, the humidity was set at 35% and the slides were left inside the humidity chamber for 1 hour after the completion of the array. Dot size was estimated to be around 800 microns. Spots exhibited no "doughnut" effect in this experiment.

Incomplete drying process



Good drying process
(37C for 1 hr)

Experiment: Methods and Results

Drying time is critical to the final quality of arrays spotted with glycerol. Arraying performed on BioDot AD5000 equipped with one dispensing channel. One Nunc Maxisorp plate was arrayed with 10 nL dots of an HRP conjugated commercial mouse antibody in a PBS/glycerol print buffer. The size of each dot was estimated to be around 550 microns. The humidity was set at 70% during the arraying to avoid static effects. Imaging was performed by chemiluminescence after adding luminal to the plate.

Conclusion

The effectiveness of any protein arraying experiment is dependent on the interaction of a variety of components of the system, including the dispenser, the protein, the substrate, the binding chemistries/buffers, and the environmental and handling conditions.

The superiority of the printing method offered by BioDot lies in its consistency of printing for a wide range of heterogeneous samples. While contact printing methods may deposit dot volumes as low as 1 nL, with diameters in the order of 75 microns, contact dispensers are often limited by the viscosity of the solution and by clogging issues. BioDot's BioJet Plus non-contact, microsolenoid-based dispensing system, and the Scienion piezo-electric based system can deposit dots of 0.3-5000 nL, with CVs below 10%.

In considering arraying systems, the user must keep in mind a variety of factors, as discussed above. Key among those considerations is the scalability of the manufacturing process, which is a reflection of the design and manufacturability of the product. Working with equipment manufacturers from an early stage of the product design is crucial to ensuring that process requirements and equipment capabilities match..