

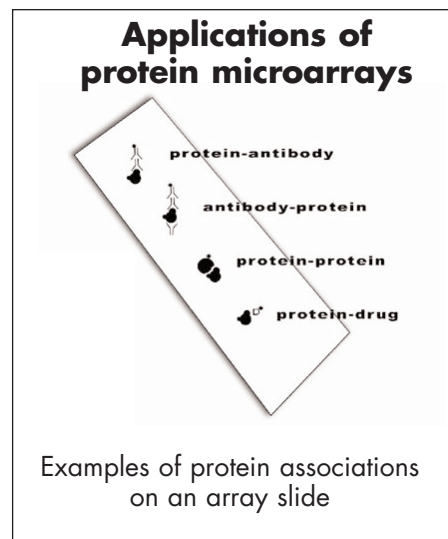
Setting Up and Running a Protein Microarray Core Facility

MICROARRAY MANUFACTURING IS AN AREA THAT IS PLAGUED WITH NUMEROUS TECHNICAL CHALLENGES DUE, IN PART, TO THE COMPLEXITY OF THE SYSTEMS INVOLVED AND TO THE VARIETY OF PROTEINS AND POTENTIAL ASSAYS USED.

The development of manufactured protein arrays is currently a hot topic because of the existence of an immense field of applications, including biosensors, diagnostics applications such as serum-based diagnostics, and pharmaceutical target design. The latter typically involves the study of protein targets through protein-protein interactions, enzyme-substrate reactions, receptor-ligand interactions, and drug-target binding. Protein microarrays can also be used to miniaturize and multiplex immunoassays and have performed better than enzyme-linked immunosorbent assays in both sensitivity and quantitative range for use in immunoassays. They are particularly well suited for immunoassay screening applications, such as inflammatory cytokines, allergens, and disease markers, and recombinant cDNA library screening for drug development applications. However, the data generated by a protein microarray can only be as good as the microarray itself. The purpose of this article is to introduce the reader to the fundamentals of setting up a protein microarray facility as well as provide some advice based on our past experience.

Thoughtful process design, bearing in mind equipment limitations, is critical to ensuring a smooth transition from research protein microarrays to a scaled up production line.

Protein arrays possess very specific chemical and physical properties. They are very sensitive to temperature, pH, and ionic strength. Furthermore, the considerable heterogeneity of proteins in solution is a major challenge that limits the physiochemical setting for retention of the protein's functionality. As a consequence, current manufacturing procedures suffer from complexity and low throughput. Several key factors should be considered when embarking on the manufacturing of protein microarrays: design of the array, type of dispensing system used and its cleaning routine to avoid carry-over issues, the environment in which dispensing is carried out, appropriate choices of chip type, reagents and buffers, and imaging technology. Optimizing the process parameters to manufacture high quality protein arrays can be time consuming and costly. Thoughtful process design, bearing in mind equipment limitations, is critical to ensuring a smooth transition from research protein microarrays to a scaled up production line.



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DISPENSING PLATFORMS

Commercially available nano- and pico-liter dispenser platforms are available to perform a wide variety of applications at a variety of scales, from laboratory to full scale production. Their design must exhibit some degree of flexibility to adapt to the variety of applications of protein microarrays and should include a number of features critical to demanding processes encountered for diagnostic applications: aspirate and dispense capability, wash and dry stations to avoid carry-over issues, drop monitoring system, humidity and temperature control, flexible and easy array map software, flexibility of the number of dispensing channels, high throughput and ability to customize deck configurations for different chips.

The dispensing technology is critical to the quality of the final protein microarray. Non-contact 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. A non-contact, piezo-electric arrayer will deliver high throughput and high quality prints for high density microarrays in a picoliter range thanks to a robust technology based on fluid pressure, piezo-electric impulse, and glass capillary. Contact silicon pin arrayers are recommended for volumes below 100 pL and for applications on robust chips because their contact deposition mode may damage coated surfaces, such as three dimensional membranes that are routinely used to keep the proteins in their native folding structure.

CHIPS

Different chips carry different surface chemistries which make them appropriate or not for different applications. When performing antibodies assay, or when doing interaction studies, a directional binding is important, such as the one present with tagged surfaces Ni(II)-NTA or streptavidin. To keep the native protein structure in its original 3-dimensional fold, surface modifications are necessary, such as hydrogel™ or nitrocellulose and zeta-grip™ membranes. High protein binding, 3-dimensional chips like zeta-grip™ are available that provide a porous 3-dimensional environment that protects many epitopes. This translates into better sensitivity and assay performance. These are particularly well suited for colorimetric applications but work with fluorescence as well. They have very low background and very high signal to noise ratio but are not compatible with detergents. For covalent protein binding, additional modifications are required, such as aldehydes, amines, and epoxides. Generally glass slides have a 2-dimensional

binding surface and are of limited value in protein microarray studies.

Distinct surface chemistries will be responsible for different spot sizes and morphology of the protein microarrays, causing weaker signals on the detector and possible coalescence and splattering occurrence. Identification of the best possible substrate for a specific protein arraying process is critical to success for manufacturers of protein microarrays.

ARRAYING BUFFER CHOICE

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 and for one type of protein array. Buffers such as PBS, Tris, MOPS, and Hepes will provide good arrays for soluble proteins but for insoluble proteins special buffers are required, such as acetic acid buffer for collagen. Optimization of the arraying buffer often turns out to be a tedious task bearing lots of compromises. To a lesser extent, 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, while protein array buffers are typically designed to enhance protein stability and signal intensity. The spot roundness and size for protein microarrays are highly depending on the arraying buffer.

CARRY-OVER ISSUES

When defining a cleaning strategy, it is important to consider the particular characteristics of the proteins arrayed and to choose cleaning reagents appropriately. Certain proteins or protein diluents may be incompatible with water and prefer organic-based cleaning solutions. Cleaning of proteins from dispensing tips can be achieved using a simple aspirate-and-dispense wash strategy. In high throughput, non-contact arraying, dispensing microdroplets (nL to µL) of concentrated aqueous solutions of protein can cause blockage of ceramic tips with subsequent damage. 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. Accuracy, precision, production costs, and rates 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 non-contact approach, production time allotted to pin cleaning can exceed the arraying period.

FLUID DEGASSING

Non-contact dispensing technology exhibits a 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 a continuous column of fluid inside the dispensing channel — meaning in the absence of any air bubbles. De-aeration of all 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.

An innovative method to achieve efficient degassing is offered by a flow-thru vacuum degassing chamber. This chamber contains a single amorphous per-fluorinated copolymer (Teflon® AF) degassing membrane. It comprises a continuously vented mini-vacuum pump with a unitary PTFE diaphragm. This efficient degassing method reduces the dissolved oxygen inside fresh water from 8 ppm for a fully aerated solution to 1 ppm after passing through the degassing chamber. Several vacuum degassing modules may be mounted in parallel on one arrayer. This degassing method eliminates any set-up time required for degassing prior to any dispensing experiment.

ENVIRONMENTAL CONTROL

Both during and after arraying, it is crucial to maintain a controlled environment. Cleanrooms 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 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. Moreover, the drying time is considerably increased by the addition of glycerol. Drying slowly the protein array in a controlled manner usually works well to avoid the “doughnut effect.”

DETECTION

There are two primary ways to detect protein microarrays: fluorescent and colorimetric. Fluorescent detection of protein arrays resulted from translation of nucleotide array technologies to protein microarrays. They generally have a large dynamic range but limited sensitivity because they are molar based (not enzyme amplified). Enzyme-amplified or “colorimetric” arrays result from a translation of traditional immunoassays and hence are more directly comparable. The primary advantage of performing the chemistry on the surface of the array is the increased signal density. For example, if an enzyme produces X product over volume Y, in the case of ELISA, Y is a large volume, typically 100 μ L. However, for the colorimetric array, Y is a low volume and may be less than 0.01–1 μ L. Since the signal intensity equals X/Y, the microarray is effectively 100–1000 times more sensitive than ELISA. Fluorescent protein microarrays are reported as having sensitivities equal to ELISA, probably because there is no amplification of signal.

Fluorescent protein microarrays require a fluorescent detection device. This type of scanner typically represents an expense close to the cost of the microarray printer (in the tens of thousands of dollars range). Colorimetric microarrays use a high-quality photo scanner (~ \$200). This reduced cost often makes colorimetric chemistry a good choice for setting up a system and testing the printer. Assays that are developed using a colorimetric system are easily transitioned to fluorescent by switching the conjugate used in the last step. This approach allows printers and assay to be tested rapidly and at a reduced start-up cost. Users may choose to then label their assay with a fluorescent label and then contact a fluorescent scanner manufacturer and have them scan it as part of a free demo. The data between the two systems can thereby be evaluated without buying the expensive fluorescent scanner. As stated above, fluorescent detection provides a greater range of detection but enzyme-based detection provides sensitivities estimated to be 100–1000 times more sensitive.

Quantification is achieved by saving the scanned image obtained from a flatbed or fluorescent scanner as a 16 bit Tiff file. This file type is accepted by a number of quantification packages developed for microarrays. Several free “share ware” quantification programs are available including “Image Tool” available free at <http://ddsdx.uthscsa.edu/dig/itdesc.html>.

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. 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.

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.

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