

Microarrays - Solutions to the Protein Challenge

Digilab Technical Note

ABSTRACT

The study of the proteome presents many challenges to the researcher. One of these is finding the best way to study tens of thousands of proteins in as short a time as possible. With the advances in miniaturisation and automation over the recent past, several approaches are now available on the market for producing high density protein arrays on specialist slides. After the arraying process is complete, lessons learnt in the automated hybridisation and imaging of cDNA and oligonucleotides now allows the consistent high throughput study of antibody / antigen interactions.

OVERVIEW

Protein microarraying is the natural progression from present DNA applications already widely used and accepted in the biological community. Many scientists have already made, or are starting to make the transition from genomics to proteomics with extremely promising results. It has taken many years to develop products of the sophistication required to address the miniaturisation and automation needs of the genomics market. These products can now be offered to the proteomics researcher because suitable adaptations have been made to respond to their specific research challenges. Further to this, there are still issues of experimental design and sample handling that also need addressing.

Current microarray technology allows for simultaneous investigation of thousands of parameters within a single experiment. Sample molecules are immobilized in ordered grids on a solid support, and are then interrogated with the correspondingly labelled binding molecules. Scanning and reading systems based on fluorescence, chemiluminescence, mass spectrometry, radioactivity or electrochemistry is then used to detect if a complex has been formed. The development of protein microarray technology now offers an important advance for the miniaturization and automation of protein/protein studies and as a market is expected to more than double in size over the next three years.

The advancement of microarray proteomics has been delayed, mainly due to the fact that compared to DNA protein molecules are inherently more complex. DNA is built out of four different nucleotides, which generate a uniform molecule with a well-defined structure and a hydrophilic, negatively charged sugar backbone. By contrast, proteins are made from 20 different amino acids resulting in highly diverse molecules with different abilities. Proteins can be hydrophilic or hydrophobic, acidic or basic and post-translationally modified (glycosylation, acetylation or phosphorylation).

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CHALLENGES TO THE DEVELOPMENT OF PROTEIN ARRAYS

There are four major challenges to the development of protein arrays. These are:

- 1) Antibodies are the most commonly used capture molecules, but producing monoclonal antibodies in large enough amounts is both labour intensive and expensive.
- 2) Proteins have a tendency to adsorb non-specifically to solid substrates. This can cause background problems (less sensitivity and low signal to noise ratio).
- 3) Proteins are complex, and maintaining their native state and orientation during immobilisation needs to be optimised such that their ability to interact is maintained. Also the buffers, salt, temperature and humidity conditions required for optimal activity must also be considered.
- 4) Beyond the arraying process, researchers are also keen to find ways to automate the resulting large number of antibody / antigen binding studies directly on the slide surfaces and then visualize those slides in high resolution laser scanning systems.

Finding systems that can respond to all these requirements has been difficult, but over the past ten years Digilab has developed a suite of products that can readily address the arraying, binding and visualisation needs of the proteomic market.

SOLUTIONS THROUGH AUTOMATION

Antibody Production

These challenges have been approached in several ways, for example the HiGro® from Digilab is an incubation system specifically designed to grow cultures in microtitre plates. Its small orbit and controlled temperature and gas flow results in fast cell growth and high levels of protein expression.

Protein Adsorption

Several slide chemistries are available to solve the issues of binding and orientation. These include membrane based technologies from Schleicher and Schuell (FAST® and CAST™), and proprietary surfaces such as Accelr8's OptArray™-Protein slides, NUNC™'s polymer coated glass slides and the PerkinElmer™ HydroGel™ slides. It is also possible to use existing silane, polyacrylamide, epoxy, silylated, glyoxyl, and aldehyde surfaces, and the newer nickel and streptavidin coated slides.

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Protein Arraying

One myth regarding protein microarraying is the assumption that it is not possible to use contact printers to manufacture quality protein microarrays. This is incorrect as shown by the large and growing number of publications and commercial products that are being produced using contact printers. Indeed, the first experiments carried out in the field of protein microarrays were from contact printing instruments.

Now, most of the World's leading microarray laboratories are carrying out their research using contact printing systems. Even considering the more viscous solutions needed, both solid or split pins can be used as long as specific printing parameters, such as touch-off speed can be controlled. In addition, the incorporation of sonicating water baths does assist in the cleaning process of the pins.

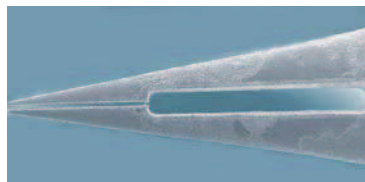


Figure 1: The Digilab Arrayers Include Soft Touch Technology, Cooled Plate Storage and Sonicating Wash Baths

An important factor to consider when setting out to array proteins comes from the choice of slide chemistry and spotting solution; and combining this with the environmental considerations has a major effect on array quality. The ability to cool the arrayer and control the local humidity is extremely significant in the production of high quality protein microarrays. Using the plate chiller in the BioBank area of the MicroGrid II system from Digilab acts to reduce the internal temperature of the instrument, and enhances successful protein arraying.

Two of the more popular slide types for protein microarray production are the FAST system from Schleicher and Schuell and the HydroGel systems from PerkinElmer. It has been said that it is not possible to use these surface types with contact printers for the microarray production. This too is not actually the case, and for use in protein microarrays, the HydroGel substrate is compatible with all Digilab microarray printing technology. The contact arrayers from the OmniGrid® and MicroGrid ranges allow the researcher to control the spotting speed (using Soft Touch with user selectable speeds) when printing on such delicate surfaces to prevent damage. Adjustable target height then allows for the differences in the thickness of the many slide types now available.

Figure 2: A selection of solid and quill pins are available for all arraying products.



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DNA/RNA Synthesis

Digilab also manufacture their proprietary synQUAD™ solenoid valve technology for non-contact printing. This has been in use for protein spot deposition for several years. Although piezo-dispensing technologies can print at higher densities, concerns remain over the heat production from the piezo crystal (bad for your delicate proteins) or blockages from viscous products. This latter issue requires highly expensive vision correction systems. SynQUAD shows robustness, reproducibility and speed.

Binding and Imaging

Once the arrays have been produced, there is still the need to study antibody / antigen interactions. The Digilab HybStation is a programmable automated hybridization and wash station typically used for cDNA and oligonucleotide microarray hybridizations. Its versatility also allows it to be used for carrying out antibody - antigen interactions. If fluorescent tags have been used, then the results can be imaged in the two laser (red/green) or four laser (red/green/blue/yellow) UC4 scanners with a resolution down to 1µm and the ability to read four slides at a time.

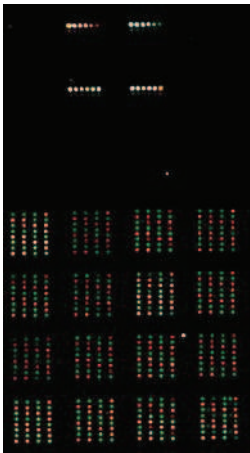


Figure 3: The large numbers of antibody / antigen binding studies generated need to be visualised and analysed.

In conclusion, there are several challenges to the production of high quality proteins and their subsequent arraying, binding and imaging. Digilab offers a suite of high throughput, automated tools to help the researcher meet the challenges associated with studying the proteome.

Worldwide Headquarters

Digilab, Inc.
84 October Hill Road
Holliston, MA 01746
USA

Phone: (508) 893-3130
Toll Free: (800) 935-8007
Fax: (508) 893-8011
E-Mail: info@digilabglobal.com

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