

Technical Note

InnoScan® 710IR Infrared detection for optimal scan of nitrocellulose-based protein arrays

This technical note is focused on the application of the InnoScan® 710 and 710IR scanners to scan protein arrays. Its principal goal is to guide customers and distributors in choosing the adequate scanner according to the microarray they want to read. For that, a general explanation of protein array construction and applications is done and some tips to choose the more appropriate scanner are discussed.

What are protein arrays?

Protein arrays, also known as protein chips, are miniaturized, parallel assay systems that contain small amounts of either purified proteins or peptides in a high-density format. Protein arrays applications go from protein profiling to the discovering of new protein-protein, protein-DNA or protein-drug interactions.

The protein array experimental bases are the same as for DNA microarrays: purified proteins, peptides or cell extracts are immobilised on a solid support, usually modified glass slides resulting in arrays of proteins. In parallel, samples such as cell extracts, serum,

tissue extracts or purified antibodies are labelled with a fluorescent tag molecule. The resultant labelled samples are then probed with the immobilised proteins on the array. If targets of the immobilised proteins are present in the labelled samples, a fluorescent signal will be detected on the array (Figure 1).

Using protein arrays, researchers can evaluate the expression and/or the activity of hundreds or thousands of proteins in a single experiment. That is why the applications of protein arrays on basic and clinical research are very large. Proteome profiling could be now possible using proteome microarrays currently existing in the market.

You can choose between the InnoScan 710 scanner for detection of fluorophores on the classical 532nm and 635nm wavelengths or the InnoScan 710IR for detection of the IR fluorescence at 785nm of excitation wavelength.



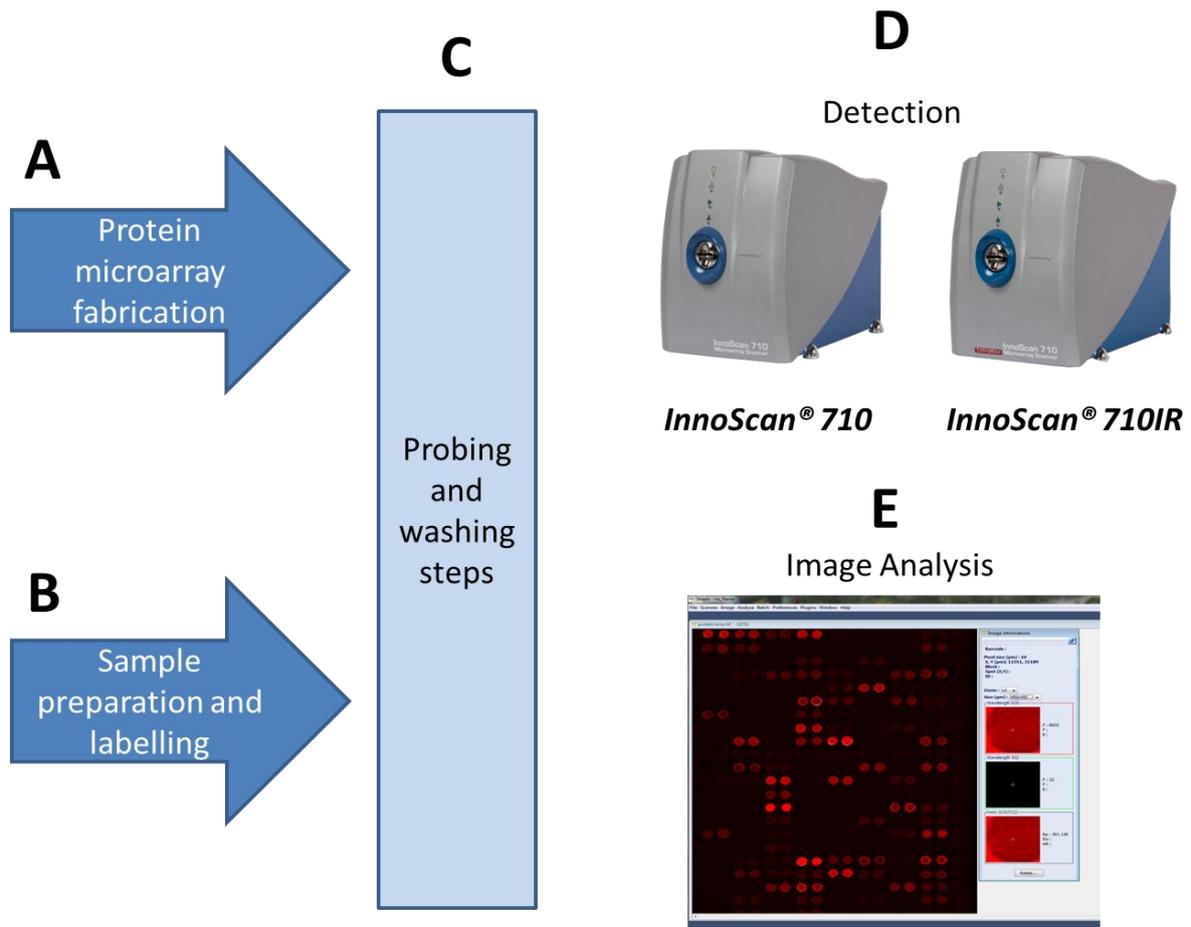


Figure 1. Protein array workflow assay

Which are the different kinds of protein arrays?

Protein arrays are classified in three groups according to the type of proteins that are immobilised on the slides and therefore their applications: *analytical microarrays*, *functional microarrays*, and *reverse phase microarrays RPPA* (Figure 2).

In general, *analytical protein microarrays* are constructed by arraying antibodies, purified proteins, well characterized peptides or non-folded antigens on the glass slide, which is then probed with a protein solution. This technique is based on the sandwich ELISA methodology and is used to measure protein

expression levels in a solution or a sample. *Functional protein microarrays* are made of full-length functional proteins or protein domains attached to a functionalized matrix usually made of a layer of a polymer that confers a porous structure. They are used for studying protein interactions with other proteins, macromolecules, or small molecules such as ligand-receptor interactions, and for testing protein activity as it is the case of kinase arrays. *Reverse phase protein microarrays (RPPA)* correspond to the miniaturization of dot blotting. In RPPA, samples such as cell lysates or plasma are spotted onto a glass slide using a robotic protein microarrayer; the arrayed samples are tested by

using labeled antibodies against the protein of interest. RPPA is a useful tool for the evaluation of protein expression levels in hundreds of different samples in a single experiment.

Which are the applications of protein arrays?

As mentioned above, the applications of protein arrays are very large; table 1 summarizes some of them. Analytical protein arrays are mainly used to establish the protein expression profiles on complex samples. In this way, researchers can profile cellular responses at the protein level face to different stimuli such as environmental stress, diseases, infection or inflammation.

With functional protein arrays, protein interactions such as protein-protein, protein-DNA, protein-RNA, protein-phospholipid, and protein-small molecule interactions can be studied. Therefore, functional protein arrays are very useful in *basic research* to study the biochemical activities of several proteins in a single experiment.

RPPA arrays are potential tools for *translational medicine and clinical research* as they can be used for disease biomarker validation and disease stratification. For example, by monitoring biomarker dynamics in response to various doses of drugs at different disease stages, researchers can associate drug efficiency at each disease stage and then defining the best therapy for each disease stage which is one of the principal goals on

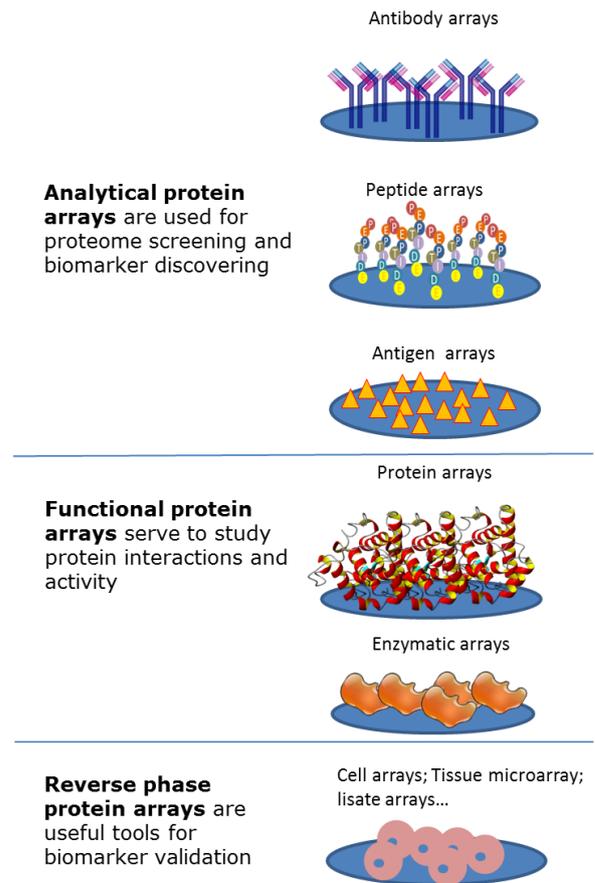


Figure 2: Different types of protein arrays

personalized medicine.

Advantages of protein arrays against classical tests for protein expression and activity

Traditional approaches for protein research allows only for one or few proteins to be studied at a time. Such as time-intensive and money-consuming assays should not be suitable for systems biology and personalized medicine purposes. Proteomics based on two dimension electrophoresis combined with mass spectrometry is laborious and time-consuming. Furthermore, as part of this methodology; proteins have to

be denatured which counteracts with protein-activity testing. Protein microarrays arise then as promising tools for elucidating the functional proteome by arraying intact proteins on glass slides coated with appropriate chemical treatments.

Why do several protein array protocols use IR labels?

One of the principal challenges on protein array technologies is to keep

protein activity intact while immobilising proteins from complex samples. Modifying the chemistry of array's supports, it is now possible to keep appropriate protein structure and activity for protein interactions assays. Advances in surface chemistry approaches have led to the development of different three dimensional supports that fit the requirements for protein stability.

Research Area	Applications
Basic research	<ul style="list-style-type: none"> ✓ Proteomics: protein profiling ✓ Antibody screening in response to disease or infection conditions ✓ To study cell signalling dynamics face to different endogenous and exogenous stimuli ✓ Discovering and screening of protein interactions : protein-protein, protein-DNA, protein-RNA, protein-lipids, protein-small molecule ✓ Sample enzymatic profiling
Translational research	<ul style="list-style-type: none"> ❖ Rapid screening of drug effect on cell signalling dynamics ❖ Biomarker validation on thousands of clinical samples ❖ Disease stratification ❖ Enzymatic activity profiling
Clinical research	<ul style="list-style-type: none"> ○ Disease stratification ○ Blood transfusion: antigen characterisation of red cells ○ Serum profiling ○ Biomarker validation

Table 1: Example of some of the numerous applications of protein arrays

Unfortunately, some of these supports give strong fluorescence signals when being read with classical microarray scanner wavelengths. This is especially true for nitrocellulose-coated glass slides which are widely used for the fabrication of protein arrays. The strong fluorescence produced by nitrocellulose is traduced in mediocre signal to noise ratio values, and therefore low sensitivity.

Using near-infrared (NIR) detection permit to decrease dramatically background issues given by nitrocellulose and other supports. Samples are labelled with a NIR fluorophore to allow target's detection. NIR detection is known to increase SNR values and highly improve sensitivity for protein interactions assays.

How to choose the more appropriate scanner version?

Scanner selection depends on the fluorophores used for labelling. Not all protein arrays protocols are designed to be read on the NIR wavelengths. Table 2 encloses flurophore compatible with the different versions of InnoScan 710 scanner.

In general, protein arrays are made of spots of sizes of more than 50 microns of diameter for which the resolution given by the InnoScan 710 scanner is largely enough. For high density protein arrays with spot size less than 40µm the high image resolution given by the InnoScan 900 scanner should be necessary.

Excitation wavelength	Compatible Fluorophores
<i>InnoScan 710</i>	
532 nm	Alexa Fluor® 555 Atto550 Bodipy® TMR-X Bidopy® 558/568 Bidopy® 564/570 Cy3 Dy®-549 Hex TAMRA
635 nm	Alexa fluor® 647 Atto647N Atto663 Bodipy® 650/665 Cy5 Cy5.5 Dy®-649
<i>InnoScan 710IR</i>	
635 nm/Emission filter 700nm	Alexa Fluor® 660 Alexa Fluor® 680 Alexa Fluor® 700 DyLight 680 IRDye® 680 Kodak X-Sight® 640
785 nm/Emission filter 800nm	Alexa Fluor® 790 Dy®-776 Dy®-777 Dy®-778 Dy®-780 Dy®-781 Dy®-782 Dy®-800 DyLigh®t 800 IRDye® 750 IRDye® 800

Table 2. Example of fluorophores compatible with InnoScan 710 and 710IR microarray scanners. This is not an exhaustive list; please contact us for further information about fluorophore compatibility.

Concluding remarks

Advances in protein array technology have permitted a rapid protein market developing. Protein array applications on disease biomarker discovering and validation reflect the

enormous potential of protein arrays on the field of personalized medicine.

In agreement with the development of new protein array technologies, Innoscan 710 and 710IR scanners bring with the high resolution and sensitivity required for this type of

assay. Using the InnoScan 710IR in combination with the appropriate infrared fluorophores, it is possible to avoid background fluorescence from supports as nitrocellulose and then to increase sensitivity which is one of the principal challenges of protein array assays□

*Dr. Adriana Lagraulet, PhD.
Field Application Scientist
INNOPSYS*

Trademarks

InnoScan is a trademark of Innopsys

AlexaFluor is a trademark of Life Technologies

IRDyes is a trademark of Li-COR Biosciences

Bodipy is a trademark of Life Technologies

Dy is a trademark of Dyomics GmbH

Dylight is a trademark of Thermo Scientific

Kodak X-Sight is a trademark of Carestream Health, Inc



Parc Activestre

31390 Carbonne – France

Tel : +33 (0) 561.971.974

Fax : +33 (0) 561.971.975

www.innopsys.fr

support@innopsys.fr