Application note:

Using the infrared detection increase SNR by 3-5 fold of nitrocellulose colorimetric assay-labeled protein microarrays.

Adriana Lagrulet[1], Mark McQuain[2]
[1]Innopsys, Parc Activestre 31300 Carbonne - France
[2]Microarrays Inc., Huntsville, AL

Introduction

Protein arrays are widely used to measure protein expression in biological samples. One of the principal challenges on protein array technologies is to keep protein activity intact while immobilising proteins from complex samples. Nitrocellulose and PDVF membranes are known due to their ability of fixing proteins while keeping their structure and properties intact. Nitrocellulose supports has been used for long time in western blot and dot blot techniques.

Protein detection methods are basically the same as those used in western blot and dot blot techniques. They can be either colorimetric, luminescence or fluorescence detection. From them, colorimetric assays are very easy to perform and very robust across a broad spectrum of users, while fluorescence detection seems to be the more sensitive method with a higher dynamique range. Unfortunately, nitrocellulose supports give strong fluorescence leading the use of fluorescence detection almost impossible at the classical 532nm or 635nm wavelengths. Here we demonstrate that using infrared fluorescence detection it is possible to increase the SNR ratio from colorimetric-labelled protein arrays. The SNR increase is mainly due to a strong diminution of the background levels.

Methods

Protein slides: Protein slides were manufactured by Micorarrays Inc (Huntsville, AL). Arrays contain conformational preserved protein antigens known to hybridize with specific human antibodies associated with a variety of auto-immune disorders.

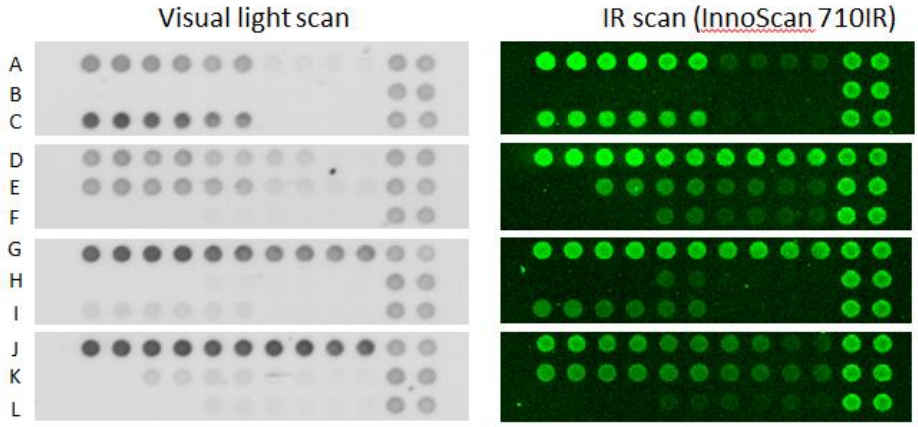
Protein detection: Probes of protein antigens were hybridized with human sera containing antibodies which were subsequently developed by secondary labeling with an anti-human antibody conjugated with alkaline phosphatase, and developed by precipitation of a NBT (nitro-blue tetrazolium) and BCIP (5-bromo-4-chloro-3;-indolyphosphate) substrate.

Slides scanning: Slides were first scanned using a visual light detection scanned and then they were scanned at the InnoScan 710IR scanner at 785 nm wavelengths (Innopsys, Carbonne-France).

Results and discussion

NBT/BCIP resulted precipitate has a large excitation/emission spectrum that permits to follow it at different wavelengths. The aim of this comparison was to show that using IR detection of a common detection dye it is possible to increase the signal to noise ratio (SNR) of the assay by 5 times. This would be translated into an increased sensitivity of protein detection.

Figure 1 shows the intensity results and the SNR ratio for each of the 11 antigens probed in the slide. The SNR was increased in average by 4-5 times when using the IR detection. This is mainly due to a diminution of the background intensity (line B).



Line	Probe	Visual light scan			IR scan		
		Feature Intensity (Average)	S/B (above blank)	SNR	Feature Intensity (Average)	S/B (above blank)	SNR
A	Antigen # 1	14520	1.6	7.2	16781	8.9	27.3
B	Blank	8896	-	-	1887	-	-
C	Antigen # 2	18711	2.1	2.6	1225	6.5	16.7
D	Antigen # 3	31633	3.6	6.5	15517	8.2	23.0
E	Antigen # 4	11294	1.3	2.8	7048	3.7	9.7
F	Antigen # 5	10887	1.2	1.0	3897	2.1	4.3
G	Antigen # 6	25911	2.9	10.5	12098	6.4	22.3
H	Antigen # 7	9972	1.1	0.6	2683	1.4	2.0
I	Antigen # 8	11175	1.3	1.6	4399	2.3	5.3
J	Antigen # 9	13291	1.5	2.9	6228	3.3	10.0
K	Antigen # 10	13687	1.5	8.1	6153	3.3	9.7
L	Antigen # 11	9154	1.0	0.6	2527	1.3	1.7

Figure 1: Feature quantification. Intensity average of each spot was quantified using the image analysis software Mapix®. S/B corresponds to the signal to background ratio where the background was calculated from empty spots (line B). SNR corresponds to the signal to noise ratio defined as:
SNR = (feature intensity mean – noise mean)/(standard deviation of noise).

Nitrocellulose has high auto-fluorescence levels at excitation wavelengths of 532 nm or 635 nm. This issue is solved when detecting spots at the near-infrared (NIR) excitation wavelength 785nm. Assuring low background levels, it is possible to increase assay sensitivity. This was the case when detecting NBT and BCIP precipitates at 785nm. Increased sensitivity leads to better detect low expressed proteins as in the case of Antigen #8 in which the SNR was increased by 3-5 fold (Figure 2).

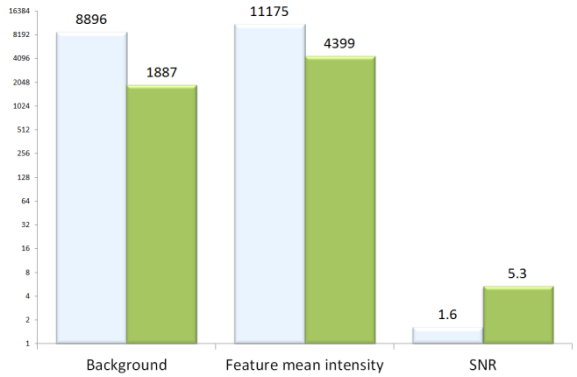


Figure 2. Feature and background mean intensity of Antigen #8. In blue: Visual light scan. In green: IR scan done with the InnoScan 710IR scanner

Conclusion

Colormetric assays conducted on nitrocellulose supports are widely used for protein detection since they are easy to perform and highly robust. Array based assays are highly multiplexed and able to query many molecular interactions in single assay that promotes automation, simplified work flow, and preserves sample. Protein arrays require low background levels to maximize detection of low concentrations of analytes in limited sample volumes.

Array based methods require a precise and accurate quantification method to objectively assess spot density over a wide dynamic range, including very small spots found in higher density multiplexed assays.

The Innopsys InnoScan 710IR scanner significantly improves the performance of this colormetric assay because: 1) It improves sensitivity by 3 - 5 fold. The IR scanning capability dramatically reduces the inherent background from the nitrocellulose. This allows low signals to be reliably detected that had previously been obscured in background from visible and fluorescent light scanners. 2) It improves quantitative resolution between different analytes. The improved dynamic range enables signals that were too close to be distinguished, to be reliably quantified and distinguished from one another. 3) It improves the multiplex capacity of the assay. The high spatial accuracy is able to accurately measure very small probe spots. This permits a greater number of probes to be included in a smaller assay well which can increase the total number of probes analyzed, reduce assay well size to require less sample, and increase the number of replicate probes to increase the statistical power of assay.

MI Microarrays Inc
HudsonAlpha Institute - Suite 3300
601 Genome Way
Huntsville, AL 35806
P: 256-327-0544
F: 256-327-0981
info@microarrays.com