Versatile and Simple Imaging of Proteome Profiler™ Antibody Arrays with FluorChem™ Imagers

Introduction

FluorChem digital imagers make it easy to get colorimetric, chemiluminescent or fluorescent images of gels, blots, plates and even colonies. And with true multi-mode capability—including visible and infrared (IR) fluorescence—on a single instrument, the FluorChem R system has all your imaging needs covered.

FluorChem imagers enable fast and easy imaging of Proteome Profiler Antibody Arrays from R&D Systems®. Quickly identify the changes of multiple analytes in your samples with one simple assay. These arrays save time and money by giving you duplicate measurements on up to 119 proteins in a single sample. R&D Systems offers 30 different arrays that cover over 1000 different analytes for human, mouse and rat. You can also use them for a wide variety of samples like cell and tissue lysates, cell culture supernatants, serum and plasma. The array kits contain all the buffers and reagents needed for chemiluminescence detection, and a simple detection antibody swap lets you do fluorescence detection on the arrays. Check out the Proteome Profiler Antibody Arrays web page to learn more about available kits.

In this application note, we'll give you tips on how to image Proteome Profiler Antibody Arrays using chemiluminescent or IR detection on FluorChem M or FluorChem R systems, and show the results from a couple of arrays.

Proteome Profiler™ Antibody Array Basics

Proteome Profiler Antibody Arrays are nitrocellulose membranes with capture antibody spotted in duplicate and buffers optimized for that specific array. For chemiluminescent detection, follow the protocol in the kit Product Datasheet and avoid mixing or substituting buffer from different array kits to maintain optimized performance. Arrays are first blocked with the blocking buffer provided in the kit, then diluted sample is applied to the array overnight at 2-8 °C. Arrays are then incubated with either a HRP-conjugated phospho-tyrosine detection antibody or a Detection Antibody Cocktail. Phospho-protein arrays detected with the horse radish peroxidase (HRP)-conjugated detection antibody can go directly to the imaging step, while arrays using detection antibody cocktails require an additional wash and incubation with streptavidin-HRP incubation. Arrays are imaged by applying Chemi Reagent Mix before imaging with a FluorChem Imager.

To visualize arrays with near-infrared (NIR) fluorescence detection on the FluorChem R, simply substitute the streptavidin-HRP with a streptavidin-conjugated fluorescent dye such as IRDye® 800 CW Streptavidin (LI-COR) diluted 1:2000 in array buffer. To avoid increased noise, we only recommend using fluorescence detection for non-phospho specific arrays where a detection antibody cocktail is used instead of a directly labeled detection antibody. You'll also want to cut off the stamped identification number on the membrane before blocking since the dye used will fluoresce and can interfere with detection. The membrane can be labeled with pencil in the empty space to the right of the array after the stamped ID number is cut off.

Imaging Arrays on FluorChem™

The FluorChem R system gives you chemiluminescent, RGB and IR detection in a single system, giving you the flexibility to image arrays using either an HRP- or fluorophore-conjugated detection reagent. If you don't require IR detection, the FluorChem E and FluorChem M systems enable chemiluminescent and RGB fluorescent imaging (**Table 1**).

FluorChem systems are fast and easy to use. Just put your ready-to-image array on a black screen with the membrane label facing up, put it in the imager, and close the door. Digital Darkroom software comes with pre-loaded protocols for chemiluminescent and IR fluorescent imaging to save you time (**Figure 1**).

DETECTION METHOD	FLUORCHEM R	FLUORCHEM M	FLUORCHEM E
Chemiluminescence	●	●	●
3-color (RGB) fluorescence	●	●	
Infrared fluorescence	●		

TABLE 1. Different detection methods available with FluorChem systems.

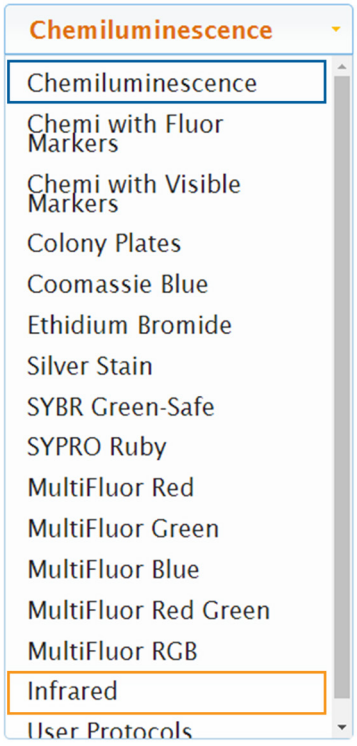

FIGURE 1. FluorChem Protocol menu with Chemiluminescence (blue) or Infrared (orange) protocols highlighted.

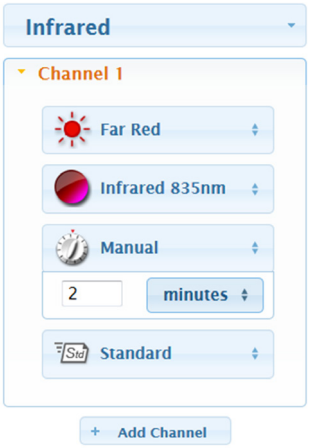

FIGURE 2. Initial exposure settings for manually detecting an array for two minutes. Exposure time can then be adjusted to optimize results and capture both low and high abundance analytes.

Proteome Profiler Antibody Arrays all contain very strong reference spots which can bias the Auto-Exposure feature in Digital Darkroom. To ensure you see both high and low abundant analytes we recommend setting manual exposure times for your arrays to optimize the signal to background levels. The image displayed on the FluorChem screen for each manual exposure is an auto-adjusted image. To best visualize the differences between the exposure times, you can view the raw, unadjusted images. Start with a two-minute manual exposure at the Standard binning setting and then optimize with a range of exposure times. Longer exposures tend to reduce background signal, making it easier to see low abundant analytes (**Figure 2**).

Analyzing Data with AlphaView Software

Images can then be exported from Digital Darkroom as a .fcz file into AlphaView® software. You'll want to visualize the images so that you see black spots on a white background. After opening the file in AlphaView, if you see white spots on a black background, invert the image using the **Reverse** tool in the Contrast Adjustments tab in AlphaView.

Use the **Rotate-Flip** function in the Enhancement Tools tab to make sure your array is level horizontally. Select **Multiplex Band Analysis** in the Analysis Tools tab. Next, select the **Region** tab and use the circular **Single Region Tool** to draw a circle around one array spot with some of the white background included. Then, select **Multi Region**

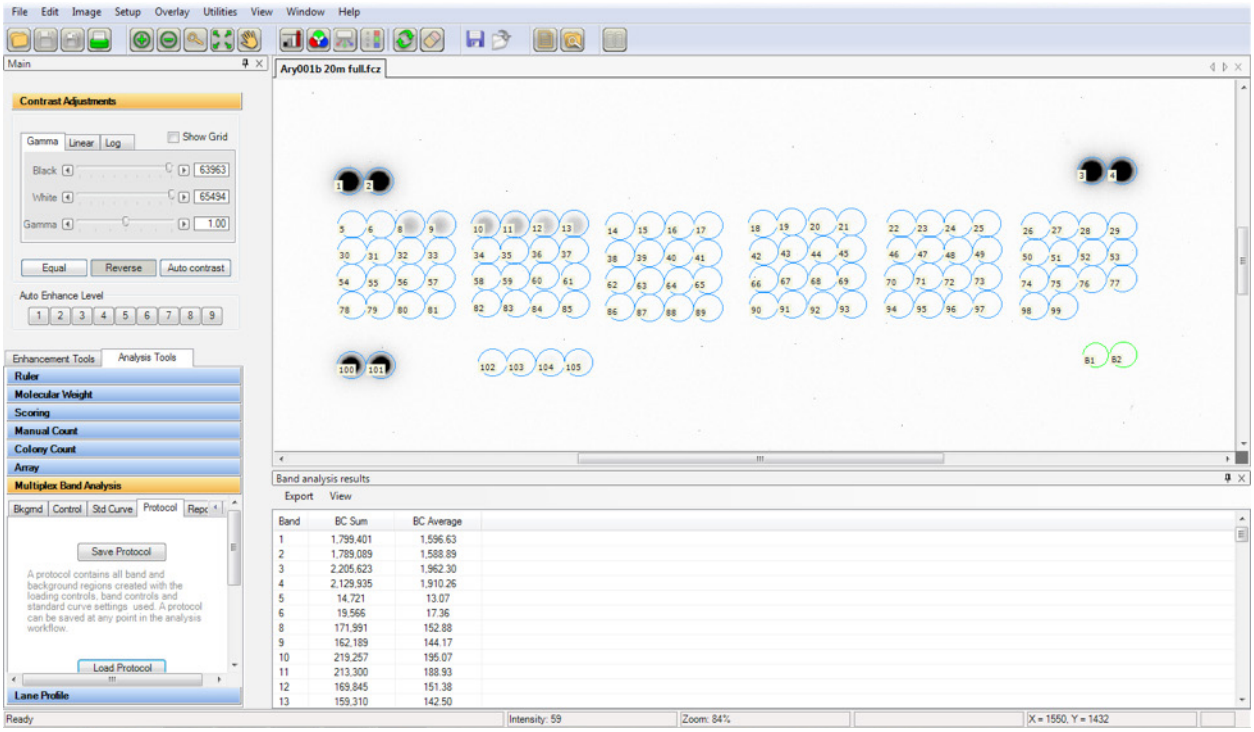

FIGURE 3. Analysis of a Human Phospho-RTK Antibody Array (R&D Systems, ARY001B) with Single Region Tool and Regional Background illustrating the selection of array spots and regional background spots.

Copy to copy the circle outline and click on each spot on the array, working from left to right then down the rows. Once every spot has been circled, deselect the **Multi Region Copy** tool and adjust circle positions so each array spot is centered. Contrast and zoom levels can also be adjusted to help fine-tune circle positions. Just take care not to change circle sizes as that affects data accuracy. Subtract background levels from your analysis by selecting the **Bkgrnd** tab and selecting **Regional Background**. Then make two circles of similar size to the analyte circle where the negative control spots are. See **Figure 3** for an illustration of analysis of an array with Single Region Tool and Regional Background.

Once the circles have been placed, click the **Export** button above the Band analysis results window, select the **Clipboard (Excel Format)** option, then paste the data into a Microsoft® Excel® spreadsheet. Values listed under BC Average represent the sum of all pixels with the average background subtracted out, divided by the area of the circle to compensate for areas of slightly different sizes. Check out Appendix B in the AlphaView Software User Guide to learn more about all the data columns available.

Select the **Protocol** tab to save the protocol as a .sda file. You can then load the protocol when analyzing other arrays. Just make sure your array images are the same size, and make sure the circles are properly aligned around each analyte spot.

Imaging Chemiluminescent Proteome Profiler™ Antibody Arrays

CHANGES IN HUMAN RECEPTOR TYROSINE KINASE (RTK) PHOSPHORYLATION

To demonstrate the data generated with a Proteome Profiler Antibody Array using chemiluminescent detection, we took MDA-MB-453 human breast cancer cells and treated them with or without 100 ng/mL rhNRG-β1/HRG-β1 for 5 minutes. We then used 50 µg of lysate on a Human Phospho-RTK Antibody Array (R&D Systems, ARY001B), and followed the protocol outlined in the product insert to screen samples against 29 phosphorylated RTK targets.

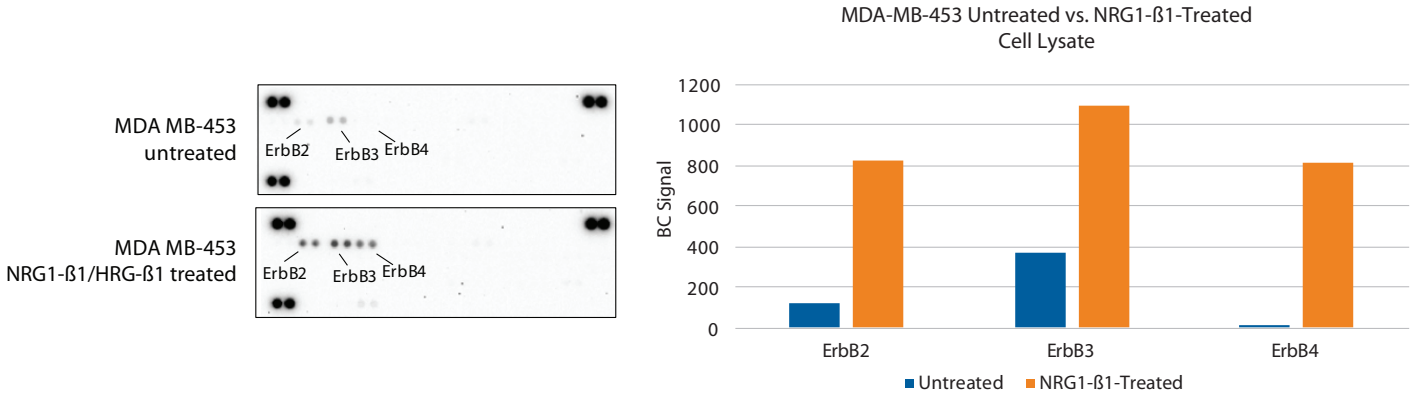

FIGURE 4. MDA-MB-453 breast cancer cell lysates treated with and without 100 ng/mL rhNRG-β1/HRG-β1 were analyzed for changes in RTK phosphorylation. Arrays (left) were imaged for 10 minutes on a FluorChem M system and AlphaView software was used to quantitate the fold increase in ErbB phosphorylation (right). BC (Background Corrected) Signal, represents the sum of all pixels with the average background subtracted out, divided by the area of the circle to compensate for areas of slightly different sizes.

Arrays were imaged on a FluorChem M system using the Chemiluminescence protocol with a series of manual scans. An increase in phosphorylated ErbB2, ErbB3 and ErbB4 was observed after treatment using a 10-minute standard exposure. The amount of phosphorylated ErbB3 increased 3.0X after treatment while ErbB2 phosphorylation levels increased by 7.0X and ErbB4 phosphorylation increased by 58.4X (**Figure 4**). These results indicate further understanding of ErbB receptors would help elucidate the effect of rhNRG-b1/HRG-b1 on MDA-MB-453 cells.

CHANGES IN MOUSE CYTOKINE EXPRESSION

Balb/3T3 mouse embryonic fibroblast cells were treated with or without 100 ng/mL recombinant mouse TNF-α for 24 hours before collecting supernatant to run on the Mouse XL Cytokine Antibody Array (R&D Systems, ARY028). 500 µL of supernatant was run on each array using the standard protocol outlined in the product insert to screen against 111 different mouse cytokine targets.

Arrays were imaged on the FluorChem M system using the Chemiluminescence protocol with a series of manual scans. TNF-α induced expression of CCL2/JE/MCP-1, CCL11/Eotaxin, CCL20/MIP-3α, CYCL10/IP-10, and TNF-α was observed using a 10-minute standard exposure (**Figure 5**). TNF-α induced expression of CCL2/JE/MCP-1 was 6.3X greater compared to untreated levels, while a 193.6X increase in CCL11/Eotaxin expression, a 991.9X increase in CCL20/MIP-3α expression and a 352.9X increase in CXCL10/IP-10 expression was also observed in the same array.

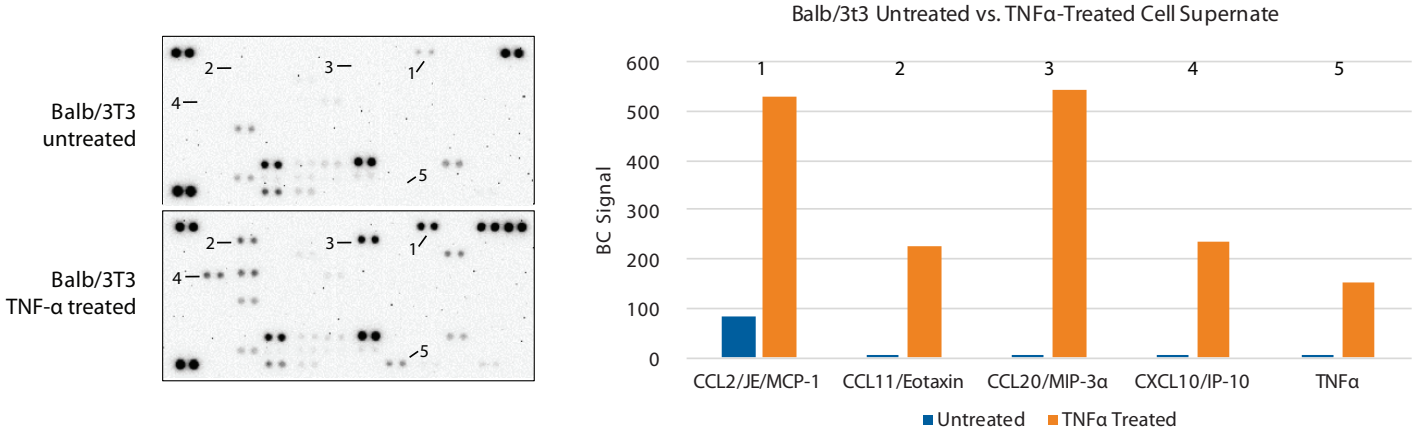

FIGURE 5. Supernatant from Balb/3T3 embryonic fibroblasts treated with and without 100 ng/mL recombinant mouse TNF-α were analyzed for changes in cytokine expression. Arrays (left) were imaged for 10 minutes on a FluorChem M system and AlphaView software was used to quantitate the fold increase in five different cytokines (right).

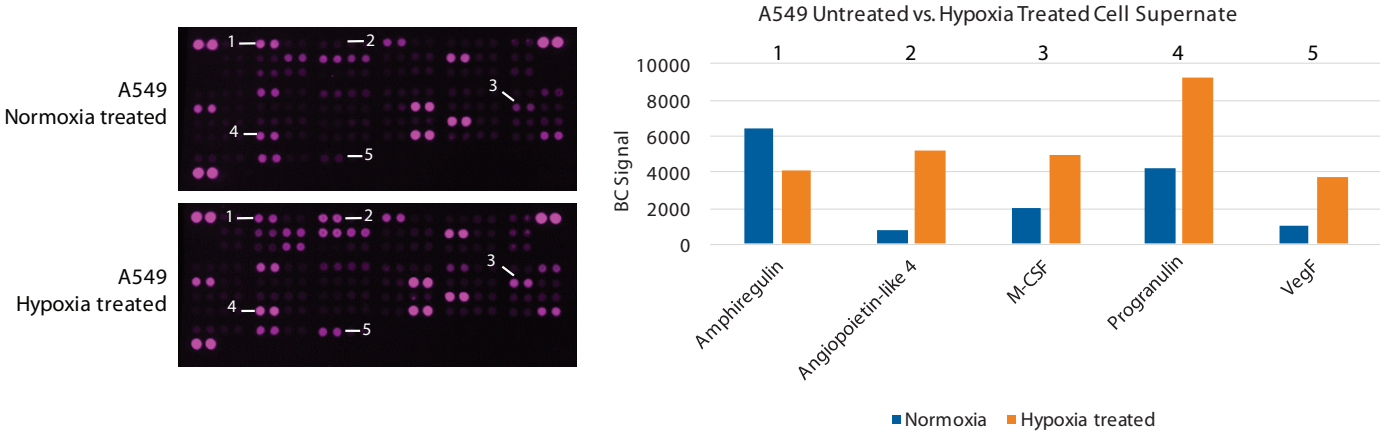

FIGURE 6. Supernatant from A549 human lung cancer cells grown under normoxic and hypoxic conditions were screened for changes in oncology target expression levels. Arrays (left) were imaged for 10 minutes on a FluorChem R system and AlphaView software was used to quantitate the fold change in five different known oncology-related targets (right).

Imaging IR Proteome Profiler™ Antibody Arrays

ONCOLOGY TARGET CHANGES DUE TO HYPOXIC CULTURE CONDITIONS

A549 human lung carcinoma cells were cultured in normoxic or hypoxic conditions (1% O_2) for 3 days before 500 µL of supernatant was applied to a Human Oncology XL Antibody Array (R&D Systems, ARY026). Samples were run on each array using the standard protocol outlined in the product insert with the exception that a streptavidin-IR dye (747 nm) was used instead of a streptavidin-HRP reagent. Samples were screened against 84 different oncology targets for differences in expression.

Arrays were imaged on a FluorChem R system using the Infrared protocol and the far IR filter with a series of manual scans. A 10-minute full scan exhibited a 1.6X decrease in the expression level of Amphiregulin when cells were grown under hypoxic conditions compared to normoxic conditions (**Figure 6**). There was a 7.0X increase in Angiopoietin-like 4 expression, a 2.5X in M-CSF expression, a 2.2X increase in Progranulin expression and a 3.6X increase in VEFG expression in cells grown under hypoxic conditions compared to normoxic conditions.

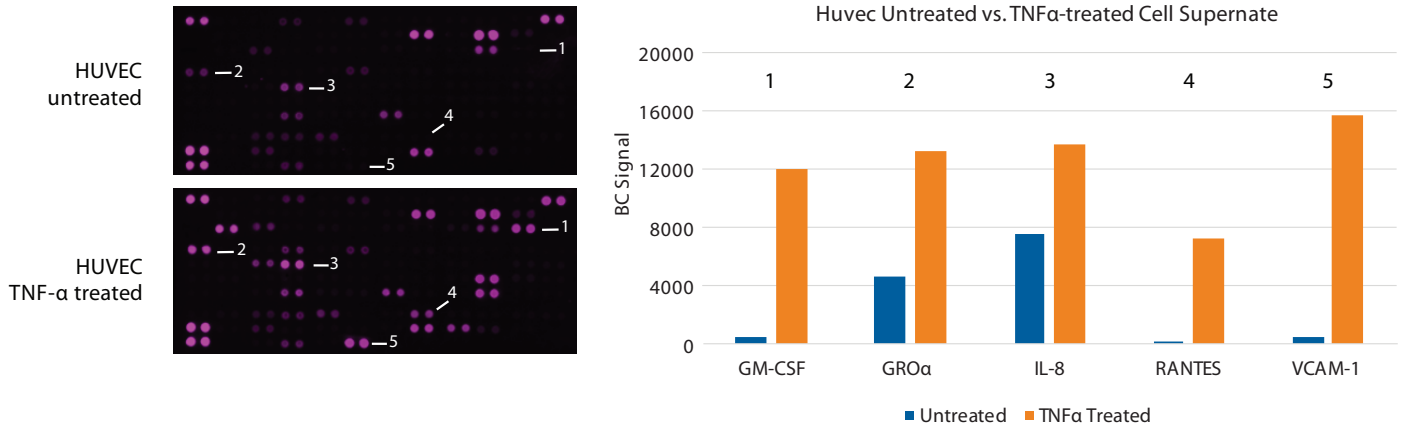

FIGURE 7. Supernatant from HUVEC human umbilical vein enthothelial cells treated with and without TNF-α were screened for changes in cytokine expression. Arrays (left) were imaged for 10 minutes on a FluorChem R system and AlphaView software was used to quantitate the fold change in cytokine expression (right).

Changes In Human Cytokine Expression

HUVEC human umbilical vein endothelial cells were cultured with or without 100 ng/mL recombinant human TNF-α for 24 hours before supernatant was collected to run on a Human Cytokine XL Antibody Array (R&D Systems, ARY022B). 500 µL of supernatant was run on each array using the standard protocol outlined in the product insert with the exception that a streptavidin-IR dye (747 nm) was used instead of a streptavidin-HRP reagent. Samples were screened for TNF-α induced expression differences in 105 different cytokines.

Arrays were imaged on a FluorChem R system using the Infrared protocol and the Far IR filter with a series of manual exposures. A 10-minute exposure demonstrated a 23.7X increase in GM-CSF expression, 2.9X increase in GROα expression, 1.8X IL-8 expression, 45.9X increase in RANTES expression and 43.2X increase in VCAM-1 expression level (**Figure 7**).

Conclusion

Proteome Profiler Antibody Arrays let you screen up to 119 targets at a time using either chemiluminescent or IR detection. FluorChem systems give you the flexibility to image these arrays with multiple detection methods. To demonstrate how, we ran four different types of samples on four different Antibody Arrays. These screens indicated treatment-induced changes in protein phosphorylation or expression that can influence your research direction.

The Proteome Profiler Antibody Arrays imaged clearly showed expression differences from as little as 1.8X to as high as 991.9X. FluorChem imagers enable easy imaging and analysis of these arrays, while also providing flexibility in detection methods.

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