NIR RPPA detection together with dynamic range extension (XDR) function in the InnoScan 710-IR scanner makes RPPA detection cheaper by 90%

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Abstract

Reverse Phase Protein Arrays (RPPA) also named cell lysate arrays have been shown to be useful tools for biomarker evaluation in large patient cohorts. Using validated antibodies, biomarkers of cell faith can be followed in a single experiment. One of the challenges of this technology is the dynamic range, since biomarker expression levels can vary from fento-molar (fM) to micro-molar (µM) concentration in the same cohort. Several technologies have been developed to follow the expression of biomarkers. Fluorescent detection has been demonstrated to be more sensitive than colorimetric and chemiluminiscence detection. However, all these technologies have to be adapted to overcome the challenge of a limited dynamic range. One solution is to perform serial dilutions of samples. However, this increases RPPA complexity and makes data interpretation quite tedious. Moreover, serial dilutions increase RPPA costs since several slides per experiment are needed. Here we demonstrate how by using the dynamic range extension (XDR) function in the InnoScan 710-IR scanner, RPPA costs are dramatically diminished when switching from colorimetric to fluorescence detection.

Proteins are considered to be the main influencers of cell response to endogenous and/or exogenous stimuli. When a stimulus happens, the cell responds by modulating the expression of "key" proteins to change cellular functions. These "key" proteins are known as biomarkers. Reverse Phase Protein Arrays (RPPAs) aim at biomarker follow up in hundreds or thousands of samples. In the RPPA technology, cell lysates are spotted on a slide and, by using a validated antibody, the biomarker expression is detected in a very simple manner (Figure 1). RPPA is actually the miniaturization of dot blots.

To visualize proteins after immunodetection, labelled secondary antibodies can be used. Secondary antibodies can be conjugated to fluorescent proteins (fluorophores) or to an enzyme such as alkaline phosphatase (AP) or horseradish peroxidase (HRP). In enzymatic detection, when the enzyme substrate is added, either a colored precipitate is deposited on the slide (colorimetric detection) or a chemilumuniscent product is formed whose light signal is captured by an adequate detector (chemiluminescence detection). In fluorescence detection, a primary or secondary antibody labeled with a fluorophore are used directly during immunodetection. In such a case, fluorescencesignal detection can be done using adequate scanners such as the InnoScan scanners rom Innopsys. Fluorescence detection has been shown to have several advantages against colorimetric and chemiluminiscence detection; the

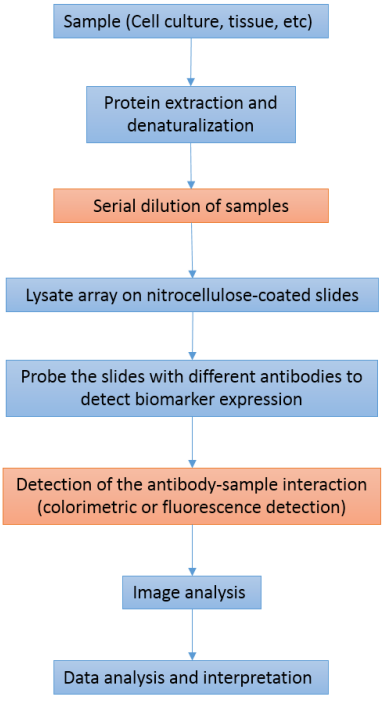

Figure 1. RPPA workflow. In orange are shown the key steps for modulating consumable costs. While serial dilution of samples increase costs; fluorescence detection with or without XDR detection diminished RPPA global costs by at least 30%.

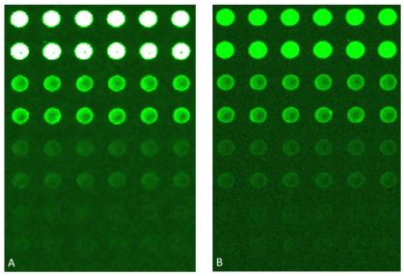

Figure 2. Dynamic range extension (XDR). In order to address the issues associated with different protein expression in samples, Innopsys has developed a scan mode to avoid saturation while keeping weak signals. A) RPPA slide scanned in a manual mode; the result image is a 16-bit TIF image with a dynamic range 4 logs. B) The same slide scanned with a XDR scan mode; the image is a 20-bit TIF image with a dynamic range higher than 6 logs.

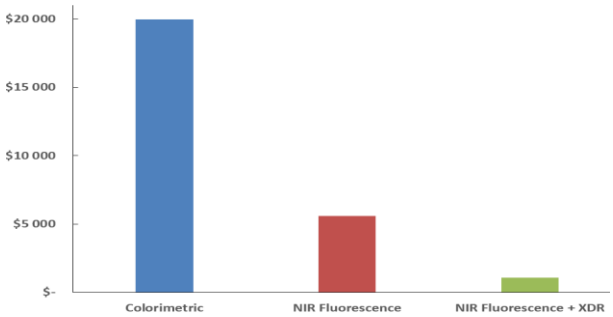

Figure 3. ***Annual consumable cost estimation***. Our reference lab estimated the annual consumable costs for 300 slides and compared between different detection methods. Costs are presented in American dollars and according to 2014-consumable catalogue pricings. A reduction of 30% of the consumable cost is achievable by using Near Infrared (NIR) fluorescence detection instead of colorimetric detection. By using NIR fluorescence detection in conjunction with the XDR scan mode, the number of slides can be divided by 3 making costs saving as much as of 90% compared with colorimetric detection.

main advantages being sensitivity and dynamic range. Fluorescence detection has 10-fold greater dynamic range over chemiluminiscence detection, and therefore, better linearity within detection limits making signal quantification more accurate.

One of the greatest challenges of performing RPPA is the dynamic range needed to detect changes of protein expression. Some biomarkers can have a dynamic range of 6 logs, which means that they can be expressed in fento-molar (fM) concentration on some samples while expressed in micro-molar (µM) concentration in others. This 6 log-dynamic range surpasses the 3 or 4 log dynamic range of detection systems. To overcome this problem, one should make serial dilutions of each sample to enlarge the detection dynamic range, but a signal amplification system has to be used to detect weakly expressed biomarkers. This procedure makes data analysis and interpretation more complex since each sample requires five or more dilution points to be included in the analysis. Also, making sample serial dilutions increases the assay cost, since the number of slides needed for an experiment are doubled or tripled, increasing the consumable cost.

Innopsys has addressed this problem by including a dynamic range extension (XDR) mode in their scanner models. Using the XDR mode, the signal detection dynamic range is extended from 4 logs to more than 6 logs thus being able to detect low and high signals in a single scan (Figure 2). The XDR mode has been shown to keep the data distribution unchangedamong different samples which is essential when comparing signals from different samples.

With the XDR function serial dilutions are no longer needed, decreasing the number of slides per experiment and hence the final costs. Figure 4 shows an estimation of the annual costs of a RPPA laboratory producing around 300 slides per year. In this example the reference laboratory has estimated that using fluorescence detection one can save around 30% on consumable costs for the same number of slides by doing the same sample serial dilutions. By using fluorescence detection together with the XDR scan mode in the InnoScan 710-IR scanner, the number of slides is reduced by 3 fold and the consumable costs are reduced by 90%.

In conclusion, fluorescence detection has been validated as a useful detection tool with several advantages: (i) Multiplexing: Using different colored fluorophores for simultaneous detection of two or more target proteins on the same slide. (ii) Sensitivity: Near infrared detection virtually eliminates background signals, thus increasing the signal-to-noise ratio by 4 fold when compared to visible fluorescence detection. It also makes the detection of weakly expressed proteins possible without a signal amplification system. (iii) Dynamic range: By using the adequate detectors such as photomultiplier tubes (PMT), sensitivity as well as dynamic range are increased. Fluorescence detection can then achieve up to a 10-fold increase in dynamic range when compared to chemiluminiscence detection. Here we have demonstrated that by using the XDR scanning mode of the InnoScan 710-IR, the RPPA experiment costs are dramatically diminished thusmaking RPPA suitable for a precise analysis of biomarkers in large cohorts.