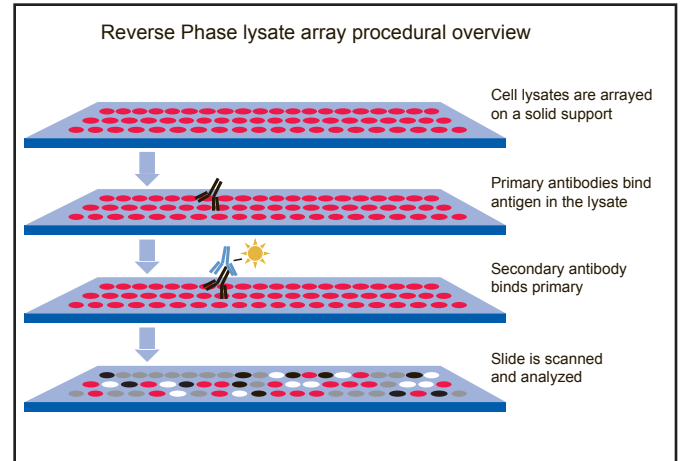


## Reverse Phase Protein Lysate Microarrays

Reverse phase protein lysate microarrays (RPAs) provide an exciting new platform for measuring protein expression levels in a large number of biological samples simultaneously. RPAs, originally introduced by Dr. Lance Liotta of the National Institutes of Health and Dr. Emanuel Petricoin of the FDA, are essentially high density arrays of micro dot-blots that can provide accurate, sensitive and quantitative protein expression data on many samples in a single experiment.

In an RPA, samples are lysed and arrayed onto a solid support, typically a nitrocellulose coated microscope slide. The printed proteins bind to the nitrocellulose and are immobilized. Slides are then probed with a primary antibody followed by a species-specific secondary antibody in a manner similar to western blotting. The secondary antibody can be labeled directly or detected indirectly using a conjugated tag. The signal from the immobilized tag can be quantified and assigned to a sample based on its physical location on the slide. A range of detection methodologies are available for RPAs such as colorimetric and fluorescent, including near-infrared. RPA data, often viewed as a series of dilution curves, is a sensitive, quantitative, and much higher throughput alternative to Western blotting.

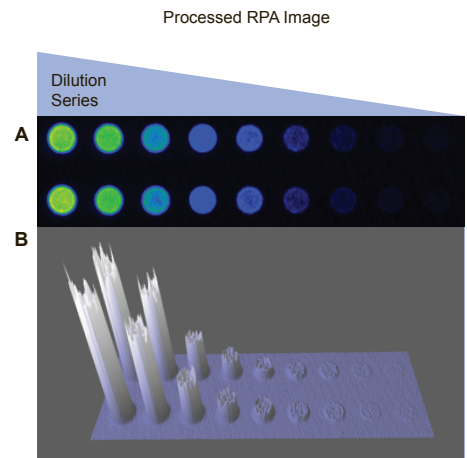


*RPA data is a sensitive, quantitative, and much higher throughput alternative to Western blotting.*

*Researchers and pharmaceutical companies are using RPAs to map protein signaling pathways, assess drug target and biomarker expression, and understand a drug candidate's mechanism of action.*

### Applications of RPAs

RPAs were first described by Paweletz et al. to measure subtle quantitative changes in multiple classes of proteins from individual cell types within tissues. Since the initial publication, this technology has also been applied to other materials including serum, plasma, and vitreous (Grote et al., Davuluri et al.). Today, there are several notable uses for RPAs such as protein monitoring for biomarker discovery and the monitoring of signal transduction proteins in response to various biological stimuli. For example, government researchers and pharmaceutical companies are using RPAs to map protein signaling pathways, assess drug target and biomarker expression, and understand a drug candidate's mechanism of action. Dr. Nishizuka (Nishizuka et al.) of the National



*RPAs are often printed in a dilution series in order to obtain quantitative data.*

*A) A cell lysate was printed in duplicate in a 10 point 2-fold dilution series using an Aushon BioSystems 2470 arrayer and probed with an anti-p53 antibody. This image is an example of Cy5 based fluorescent detection. B) The scanned array image was analyzed using MicroVigene™, an RPA analysis package from VigeneTech. The intensity of the spots in the dilution curve is shown as a 3-dimensional plot.*

Institutes of Health has created 10,000-element lysate arrays for pathway mapping, thereby permitting detailed time-course studies to be performed of protein response to cell stimulation. The ability to quantitatively track, for example, the phosphorylation cascade down one branch of a pathway versus another can guide researchers in developing compounds that best elicit the desired therapeutic effects. Clinical researchers are also examining RPAs as a potential early means of implementing personalized cancer treatment (Espina et al.). By examining the expression levels of key proteins in cancer cells isolated via laser-capture microdissection, researchers can identify the specific protein expression profile of the cancer cells. The goal of this work is to allow clinicians to recommend therapy tuned to the exact profile of a patient's cancer.

## Aushon 2470 Microarrayer

RPAs present unique fabrication challenges that are a critical problem for conventional microarrayers. Unlike genomic material, lysates are often viscous and can clog quill pin and jetting systems. Additionally, the volume of clinical samples is often low and the preferred RPA substrate, nitrocellulose, is very fragile. Aushon BioSystems' 2470 microarrayer overcomes these challenges and is a proven technology for building exceptionally high quality RPAs. The 2470 is a solid pin contact printer with unmatched versatility, able to print even the most complex biological samples onto substrates with unique shapes and chemistries as well as the most delicate of substrates, including nitrocellulose, using its soft touch deposition technology. Aushon's proprietary solid pins carry material only on the tips, eliminating the possibility of clogging. The 2470 reliably produces RPAs of the exceptional linearity and consistency necessary to permit data extraction from dilution series curves. Additionally, since there are no reservoirs to fill, the 2470 can print using very low sample volumes. These features make the 2470 the ideal choice for RPA production.



*The patented 2470 Arrayer is the industry's most reliable microarray printing platform and has been proven to be the ideal technology for building exceptionally high quality RPAs.*

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