Alignment of western blot film to silver-stained 2D gel image for HCP antibody analysis

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Overview:

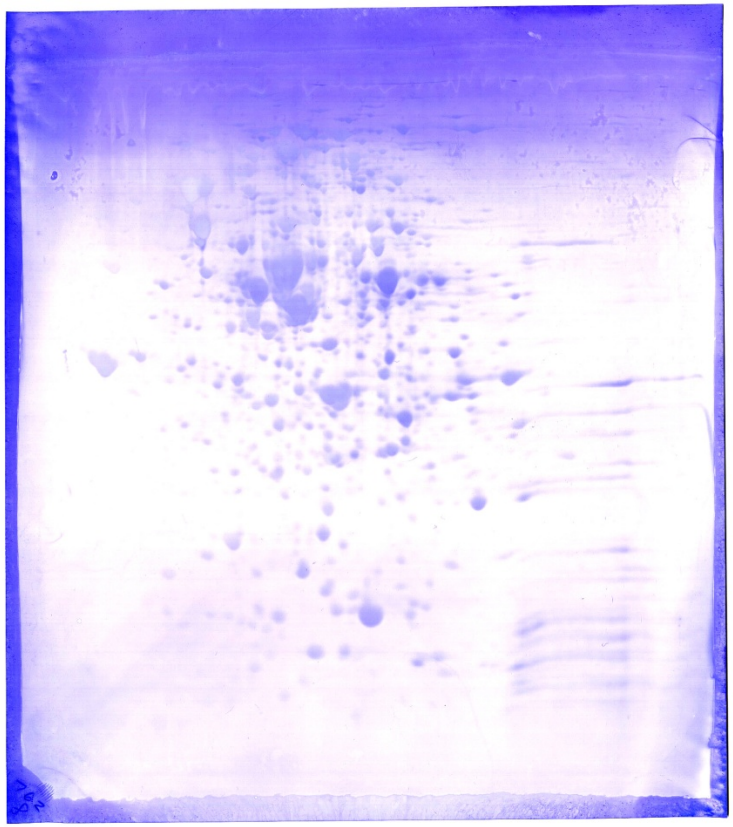
Anti-HCP antibodies used for Elisa testing are characterized at Kendrick Labs by 2D electrophoresis (2D). Total proteins in the HCP sample are visualized by silver staining 2D gels; those reacting with antibody are visualized by western blotting (WB). The patterns are matched to determine antibody coverage (percent of total protein spots detected by the antibody.)

Matching corresponding spots on the silver and WB patterns is accomplished with Progenesis SameSpots software from TotalLab. The process is made possible by Coomassie blue (CB) staining the PVDF membrane before WB.

This presentation elaborates on the process…

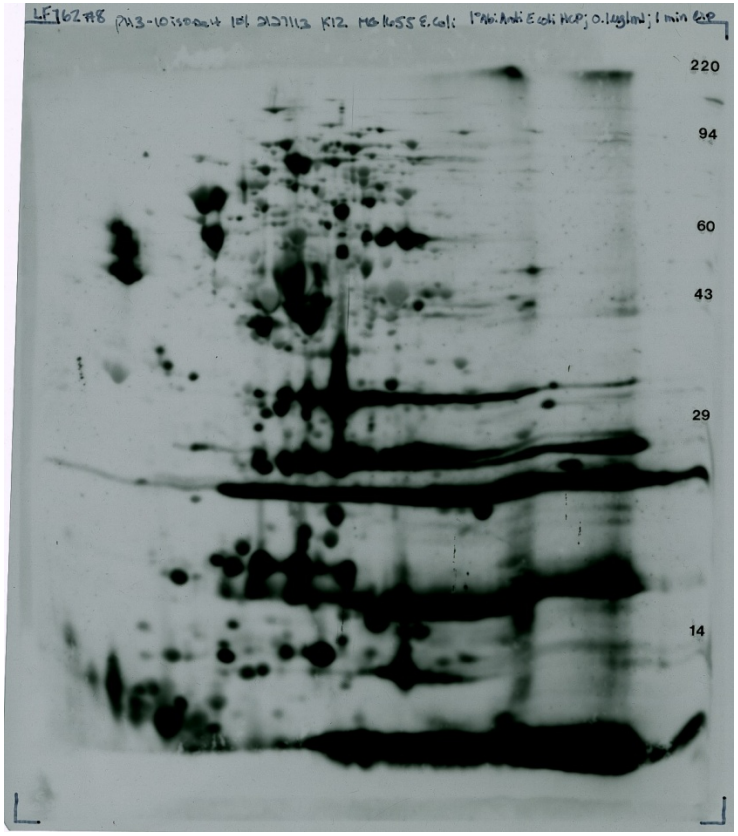
HCP lysate is run using 2DE. Duplicate gels are stained with silver. A third gel is transferred to PVDF and stained with CB before WB.

Silver-Stained 2D Gel

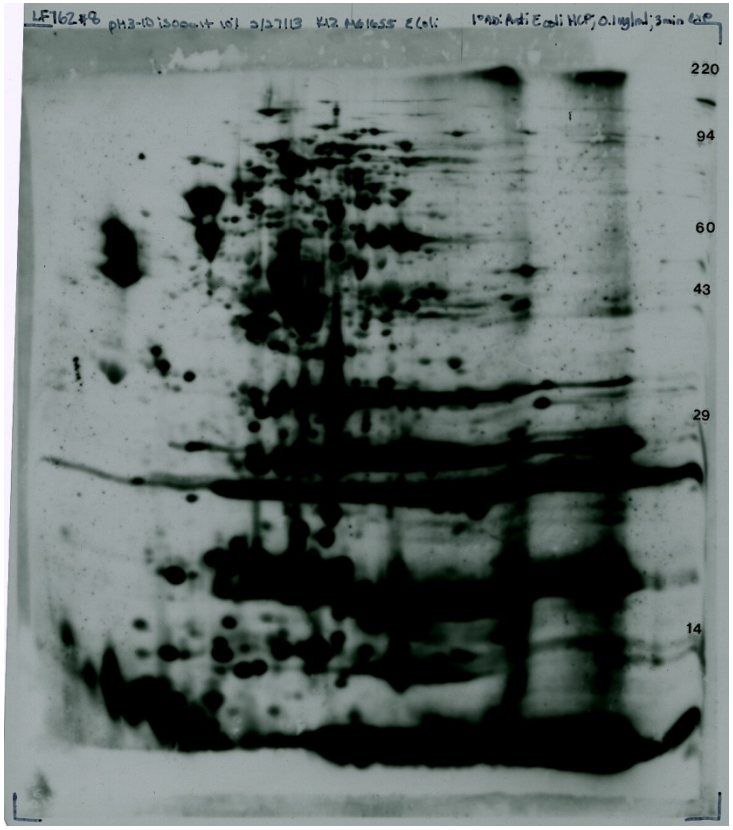


CB-Stained 2D PVDF Membrane

The PVDF membrane is destained in methanol, incubated with the anti-HCP antibody, treated with chemiluminescence reagent (ECL) and exposed to x-ray film. Multiple film exposures maximize sensitivity and dynamic range.



1 minute exposure



3 minute exposure

The western blot ECL film image is superimposed over that of the CB stained PVDF membrane

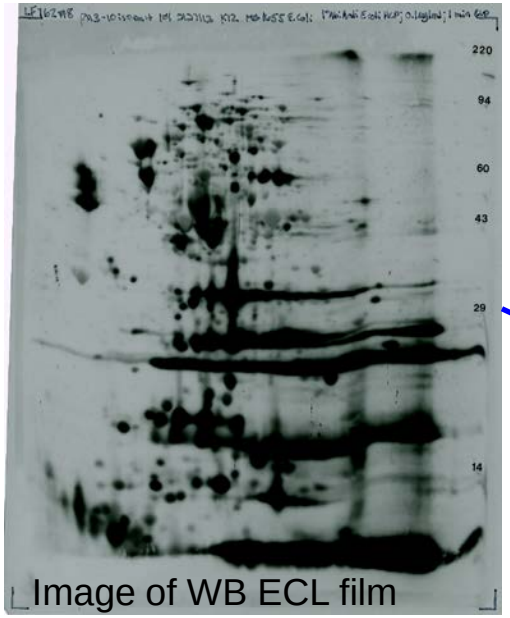
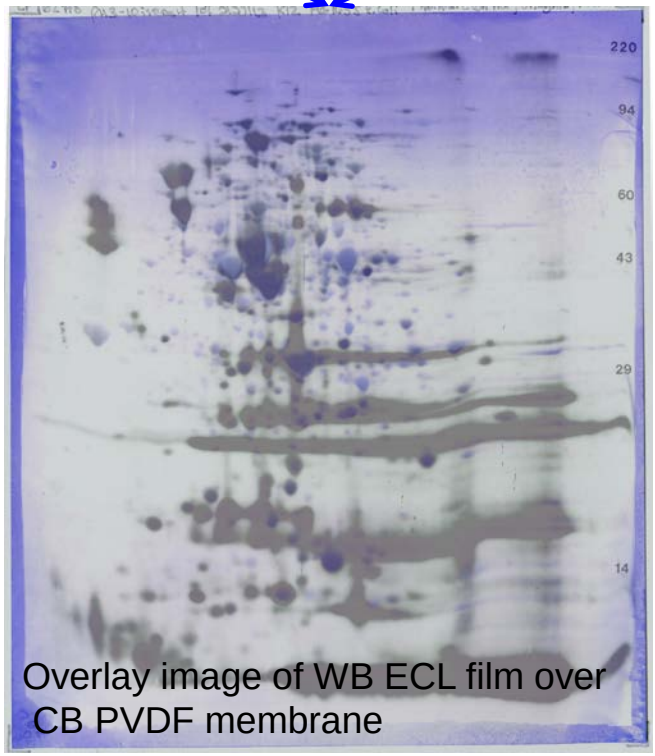


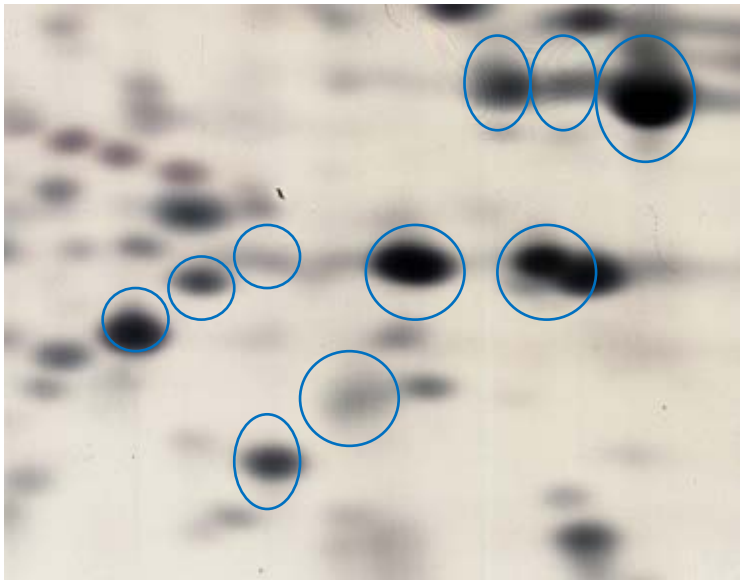
Image of WB ECL film

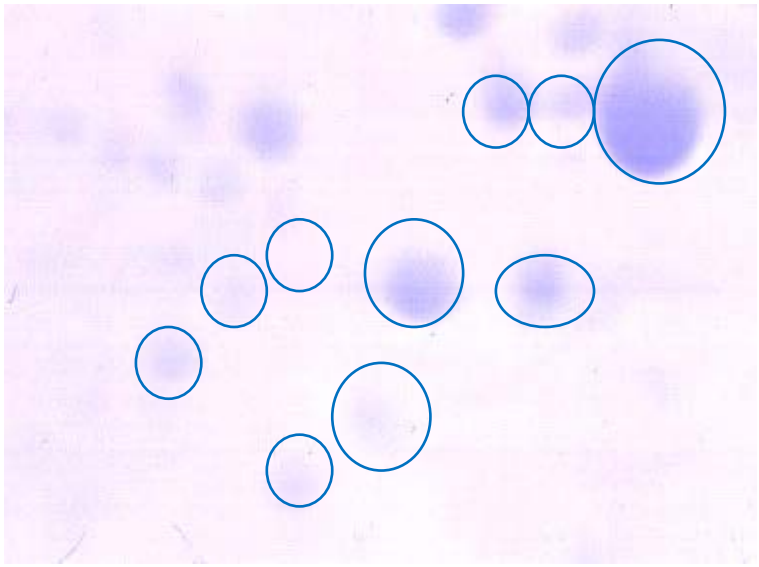

Image of CB PVDF Membrane



Overlay image of WB ECL film over CB PVDF membrane

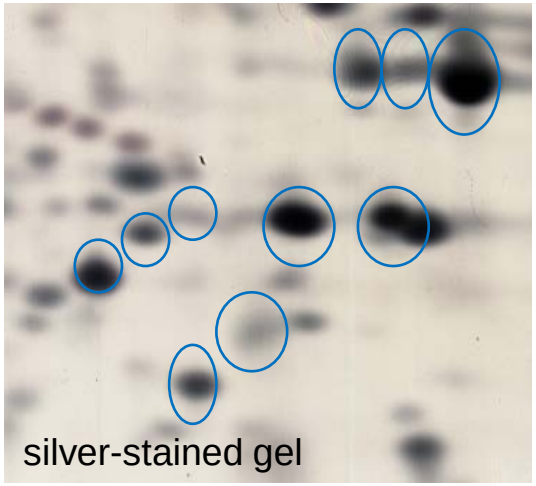
Spots detected on the silver-stained 2D gel can be matched to corresponding spots on the image of the CB stained PVDF membrane.

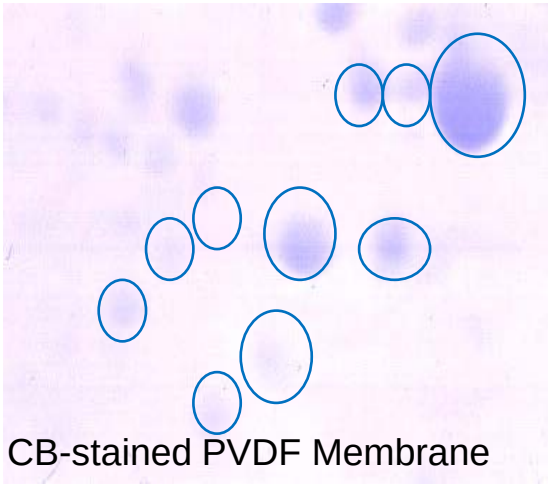


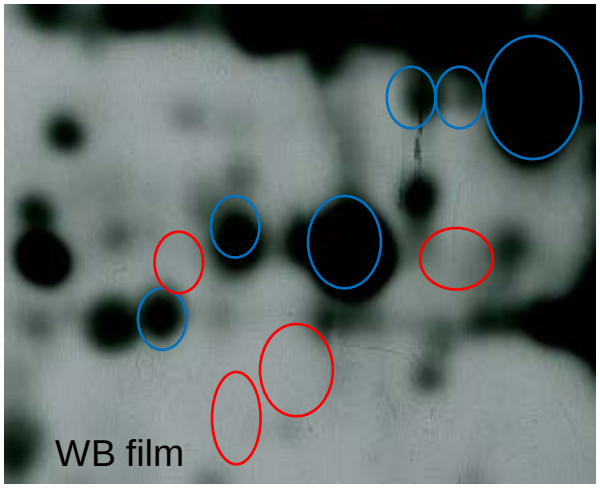


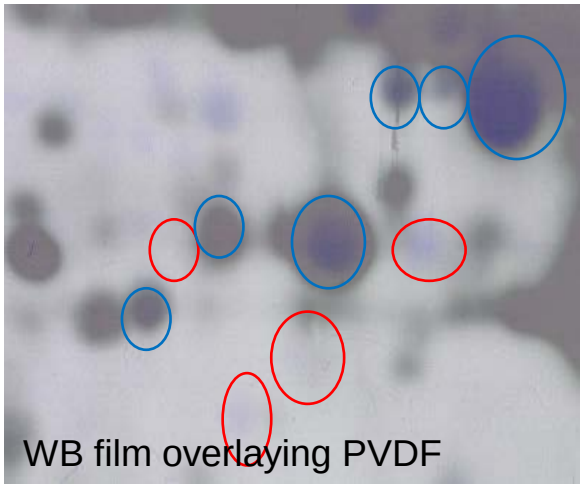
Blue outlines show example protein spots that match between the silver-stained gel and CB-stained PVDF Membrane.

Spots detected on the WB ECL film can be matched to the image of the CB stained PVDF membrane and subsequently matched to the silver-stained 2D gel.



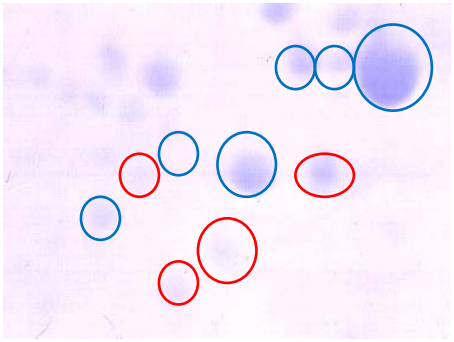


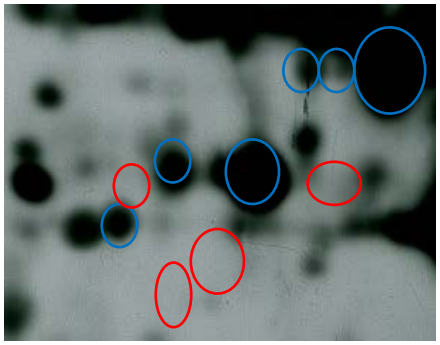


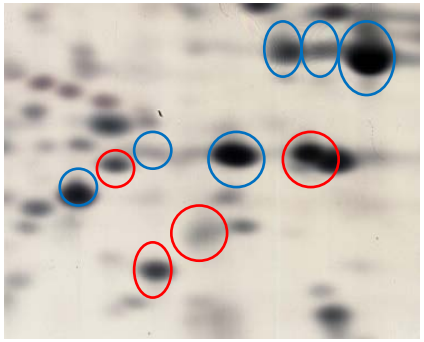


Example spots detected on the WB ECL film are outlined in blue. Spots detected by silver staining but not reacting to the antibody are outlined in red.

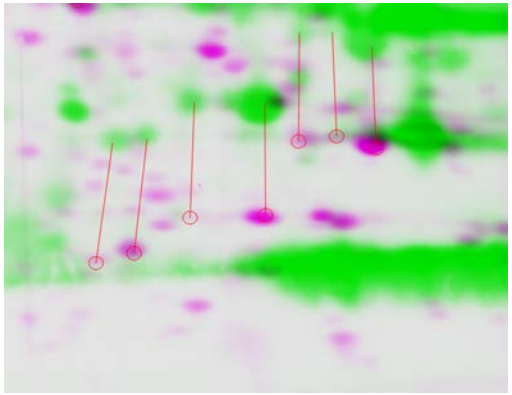
The image of the WB ECL film is then aligned to the image of the silver-stained gel using Progenesis SameSpots software.



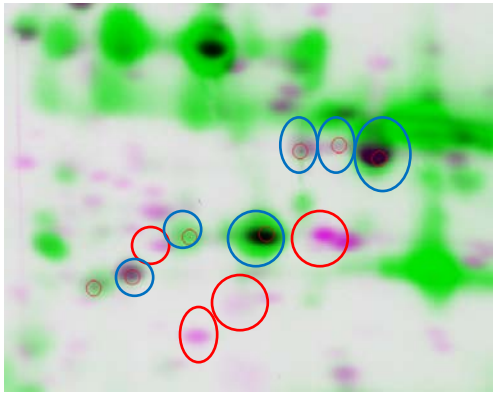




Warp vectors are generated to overlay the film (in green) exactly to the corresponding detected spot on the silver-stained gel (in magenta).

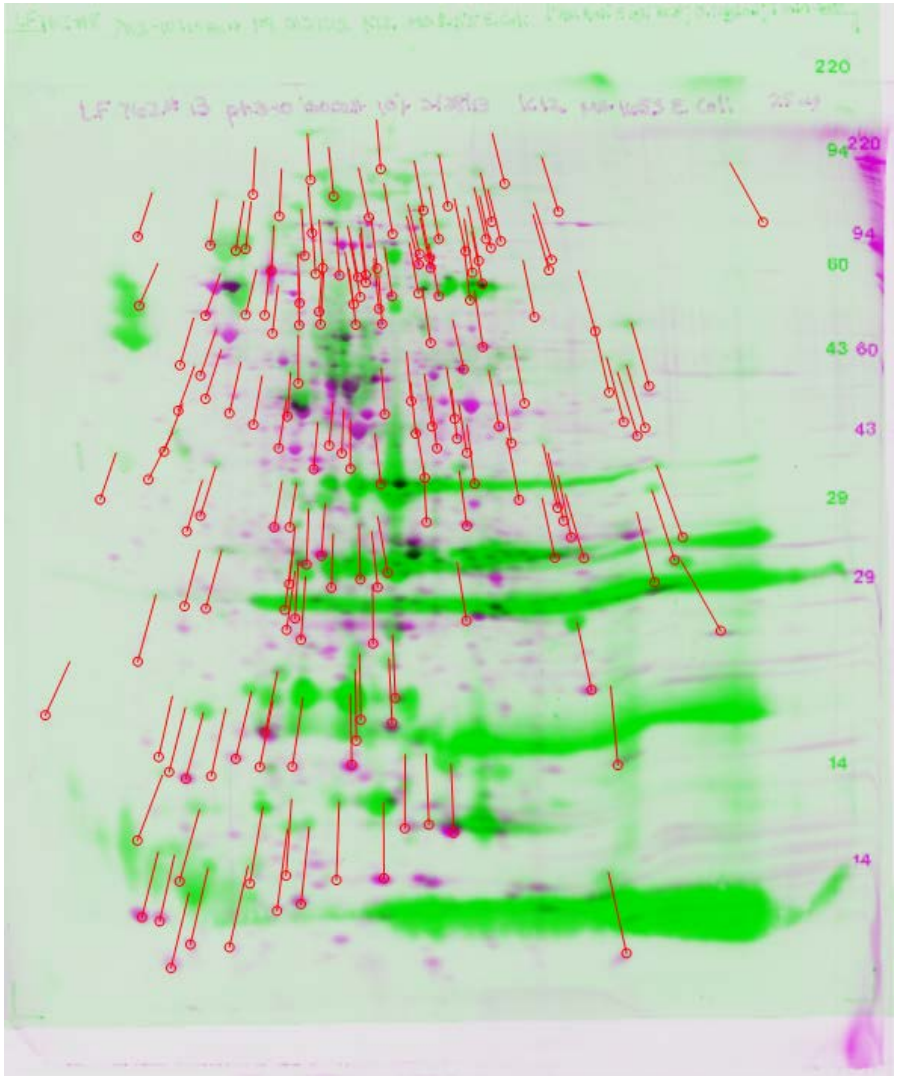


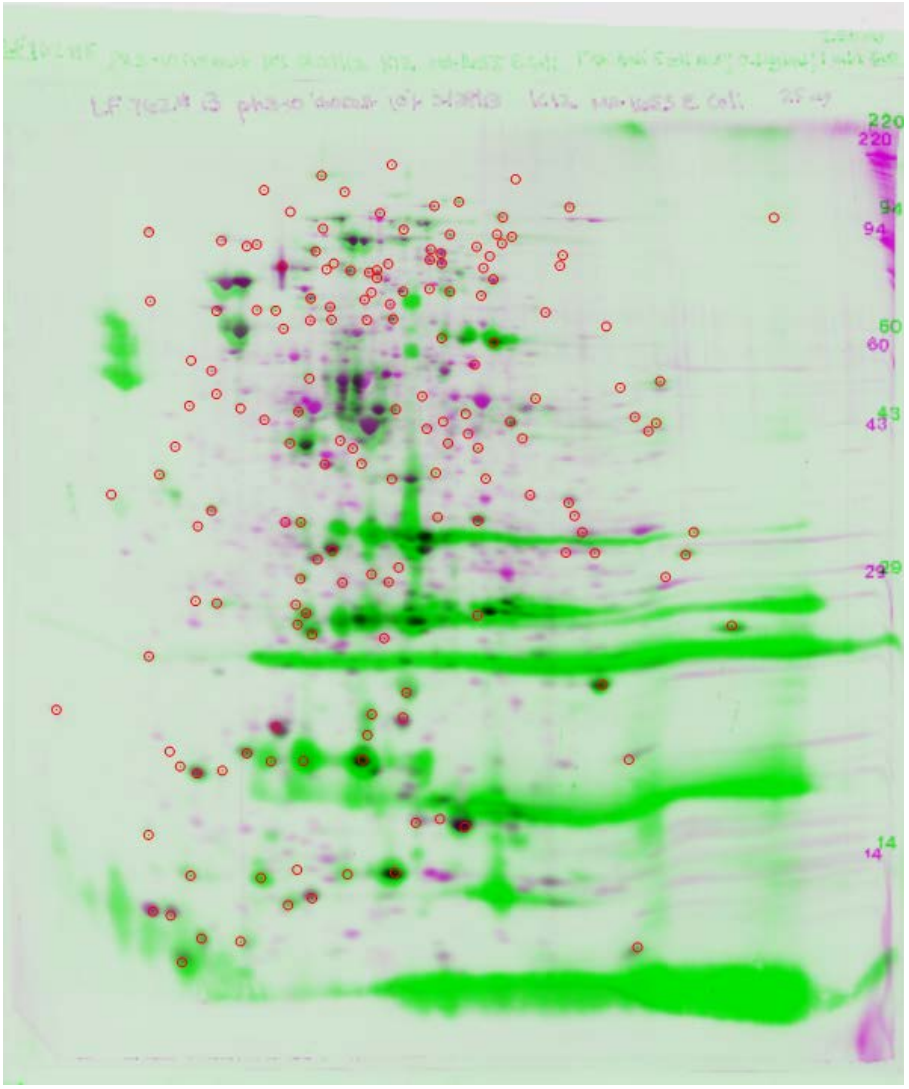
Unaligned image. Warp vectors originate at the line on the film (green) and end at the circle on the silver-stained gel (magenta)



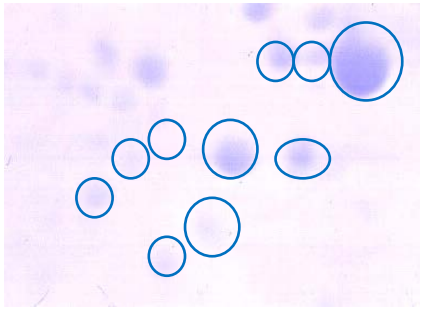
Aligned image. The film is warped to exactly overlay the silver-stained gel.

Warp vectors are generated throughout the images matching as many spots as possible until both images are completely aligned.

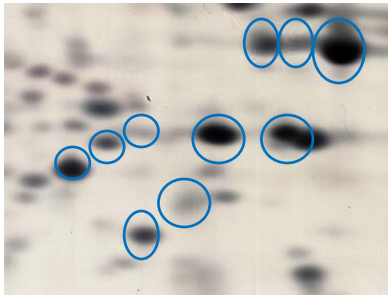




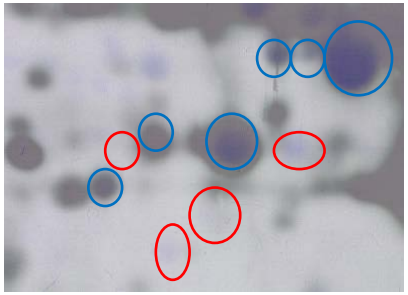
Summary:



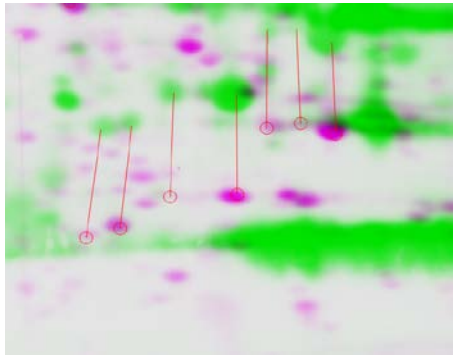
1. PVDF Membrane is CB Stained Before WB.



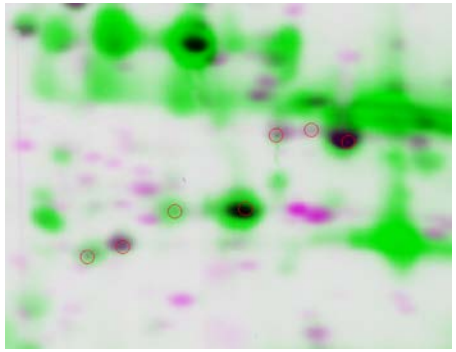
2. Spots detected on silver-stained 2D gel are matched to image of CB PVDF Membrane.



3. ECL WB film is superimposed over CB PVDF Image. Spots detected by WB can be matched to PVDF membrane and then to the silver stained gel.



4. Spots detected on the film (green) can be warped to the corresponding spot on the 2D silver-stained gel (magenta) using Progenesis SameSpots Software. Image is unaligned and shows warp vectors matching corresponding spots.



5. Image of the ECL film (green) warped to exactly align to the silver stained gel (magenta) using Progenesis Same Spots software.