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Application note

TotalLab Software Enables Enhanced 2-DE with the Auto2D® 2-D Gel Electrophoresis Device

Two-dimensional gel electrophoresis separates proteins in complex samples based on their isoelectric points and molecular weights, allowing for the simultaneous comparison of numerous proteins from complex sample types. What is often considered a laborious technique, the Auto2D® Automated 2-D Gel Electrophoresis Device is designed to simplify and fully automate two-dimensional gel electrophoresis (2-DE) for more reliable results in just 1-2 hours compared to traditional methods.

Designed with ease-of-use and consistent results top of mind, the Auto2D® device does not require advanced training to operate while also offering decreased inter-operator variability and higher reproducibility. Additionally, it provides IQ/OQ support for GMP compliance. The device has proven effective in various applications, including the analysis of differential protein expression in cancer research, disease research, separation of purified proteins for crystallization or post-translational modification analysis, and 2-D Western blotting in areas such as allergy research and cell signaling pathway analysis.

With low antibody requirements and high separation capabilities, the Auto2D® system significantly reduces the amount of time spent during sample loading, isoelectric focusing, equilibration, and SDS-PAGE. This efficiency makes the Auto2D® device unique compared to other semi-automated 2-DE systems on the market. Expanding on many of the benefits of the Auto2D® device, our collaboration with TotalLab and the coupling of their SameSpots and SpotMap software in the Auto2D® downstream workflow further enhances the previously mentioned benefits. Requiring minimal parameter setting adjustments, TotalLab software reliably demonstrates high reproducibility of spot detection when coupled with the Auto2D® device.

TotalLab Software Offers Automatic Spot Detection and Quantification

Enhanced automation and high reproducibility are achieved with the combination of the Auto2D® device and TotalLab software which improves the reproducibility and speed of 2DE and subsequent Western blot analyses. Using DIGE as the 2-DE detection method, the data below suggests that spot detection via the SameSpots software is readily achieved with samples run on gels from the fully automated Auto2D® device. Sample data is provided from gel images with spot detection results from the SameSpots software, Ver. 5.1.0.0.

The experimental materials for the data analyses include the use of extracted proteins from two types of dry yeast (Yeast A and Yeast B) using CelLytic™ Y Cell Lysis Reagent (C4482) and our Protein-Concentration Kit (2102-M). Protein was subsequently quantified with Brandford reagent (B6916) and labeled with Cy3-NHS ester or Cy5-NHS ester. Protein samples were separated on the Auto2D® device using IEF Chip pH3-10NL and 10.0% PAGE Chip while images were collected using the gel documentation system. Gels images were analyzed using the “Multiple Stains” method according to the recommended workflow analysis in the SameSpots software.

TotalLab
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Auto2D® gel images with spot detection results from TotalLab SameSpots software, Ver. 5.1.0.0

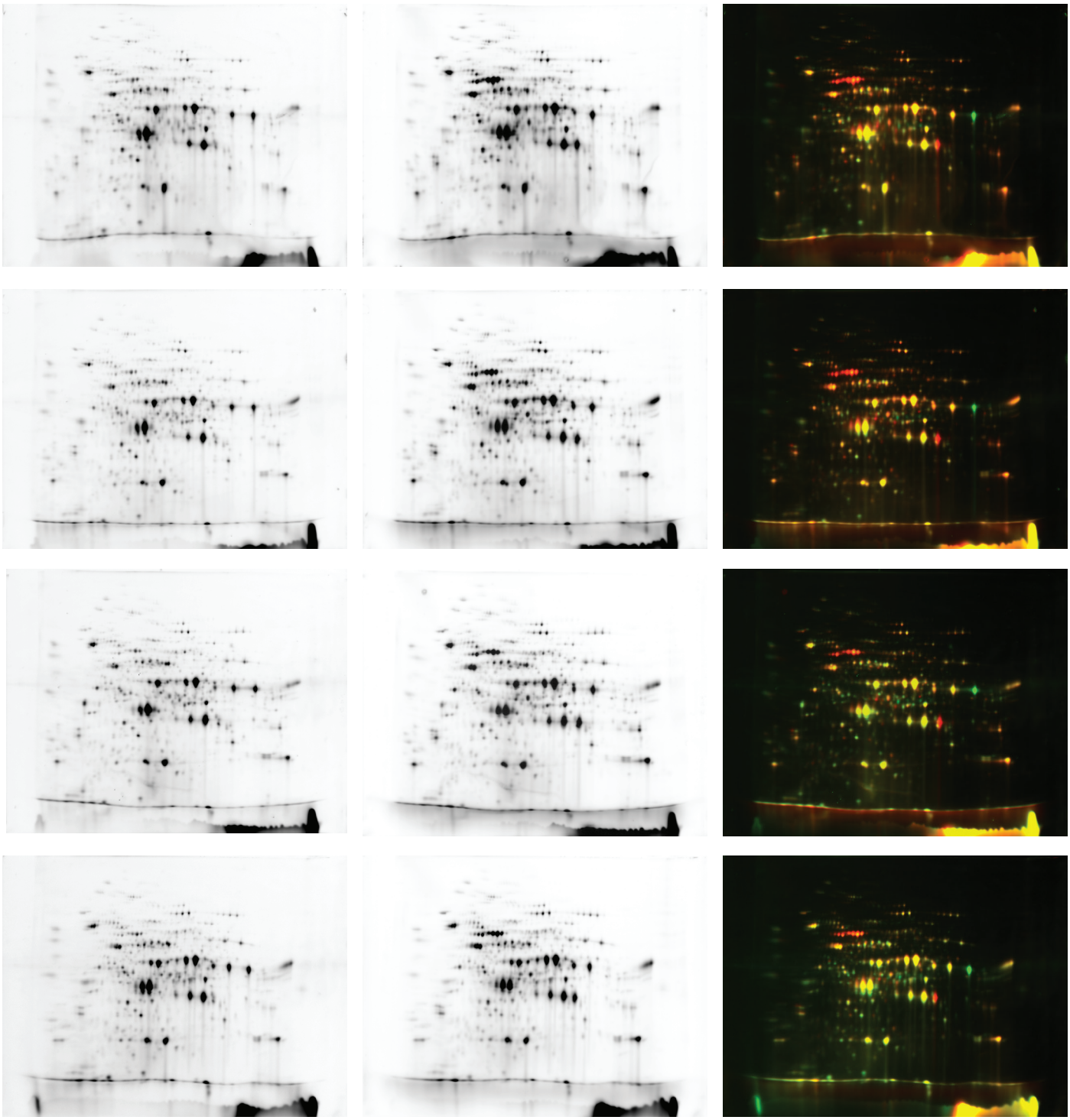

Figure 1: The 4 sets of data for yeast extract sample data sets obtained by DIGE suggests high reproducibility from the Auto2D® device.

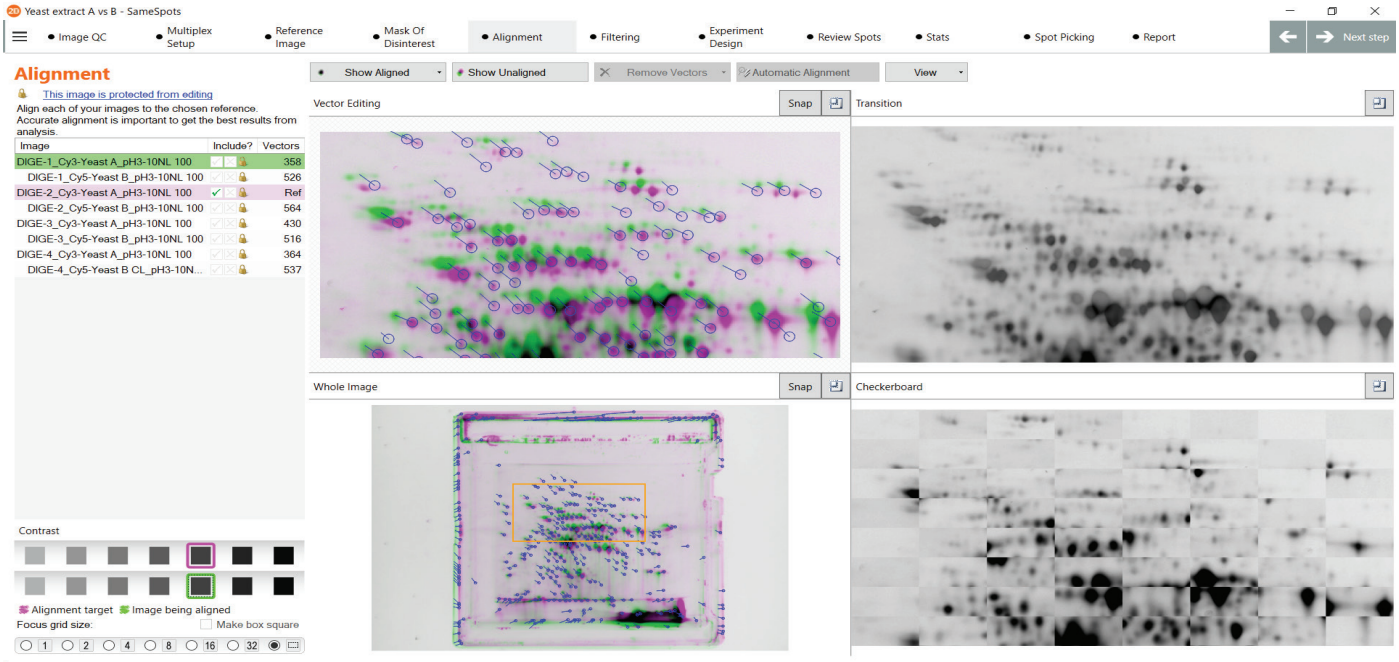

Figure 2: Subsequent analysis using SameSpots software of the gels from **figure 1** using the automatic alignment feature for spot matching without manual intervention (cropping/rotation/manual alignment) suggests robust and consistent spot detection.

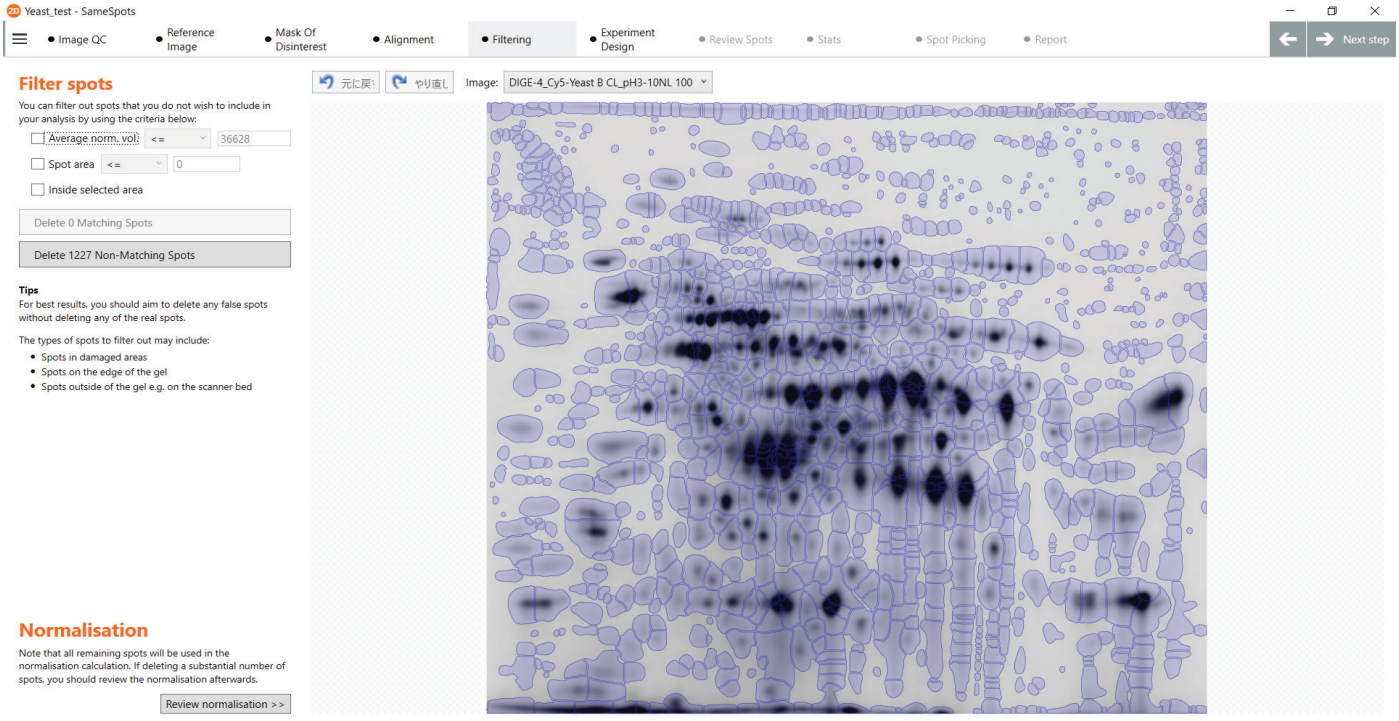

Figure 3: Numerous spots detected by the SameSpots software with only minimal manual operations necessary.

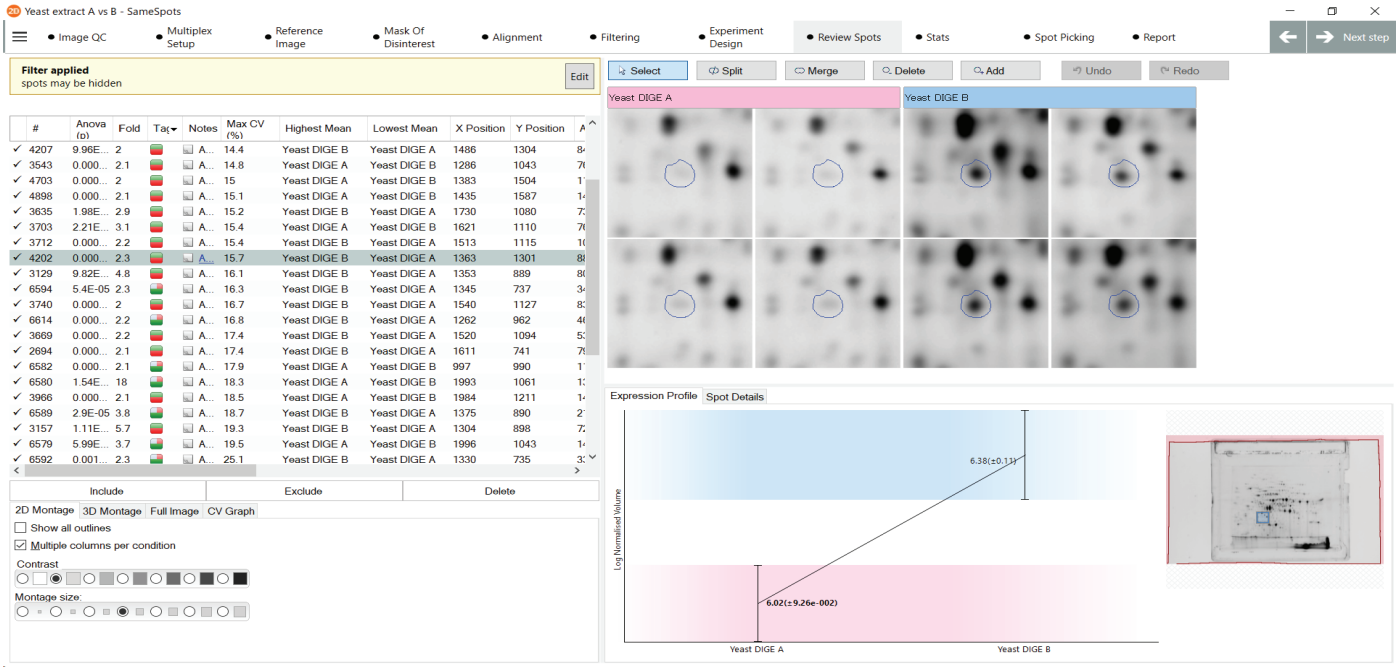

Figure 4: Demonstration of the "Quick Tags" feature in the SameSpots software that are used to identify spots with a user-configurable fold change in expression.

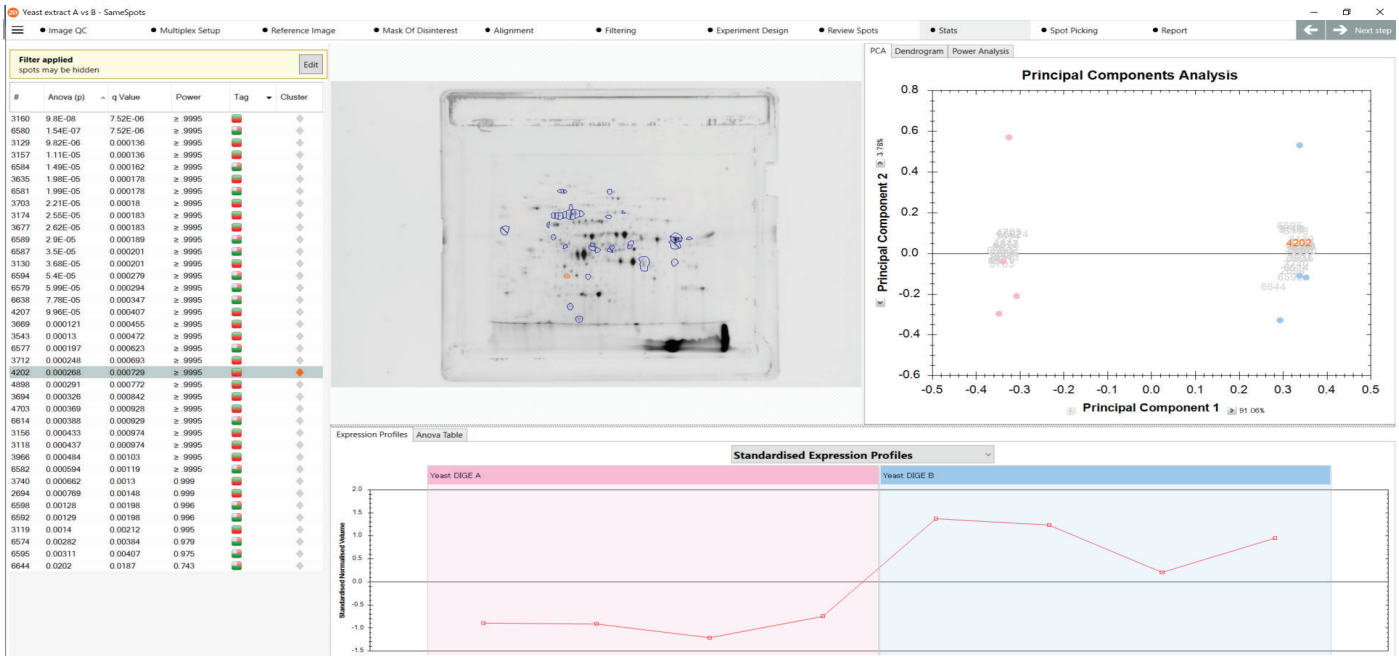

Figure 5: Principal component analysis suggests strong correlation between proteins that were upregulated in the Yeast B vs the Yeast A strain.

Spot detection analysis of Auto2D® gels using TotalLab SpotMap software Ver: 5.1.003

Alignment of spots between total protein detection on a gel and the corresponding Western blot data is often challenging as the spot detection result is inconsistent with variable intensity for each spot. With the SpotMap software, alignment is easily achieved by the auto-align and copy alignment vectors from an aligned image functionality as highlighted with the HCP coverage assay sample data below.

HCP coverage assay using the Auto2D® system and SpotMap software

The experimental materials for the HCP coverage data analyses include the use of CHO HCP protein samples that were concentrated with our Protein-Concentration Kit (2102-M) and quantified with Bradford reagent (B6916). A fraction of the concentrated protein was labeled with Cy5-NHS ester. Approximately 2 µg of the Cy5-NHS-labeled sample and 8 µg of non-labeled sample were separated on the Auto2D® system using IEF Chip pH3-10NL, 10.0% PAGE Chip, and the gel was imaged using the gel documentation system. Proteins were subsequently transferred onto a membrane using the Immobilon®-FL (IPFL00010) by fast transfer system.

Two different anti-HCP antibodies were used for the Western blot immunodetection. HCP coverage was calculated for the gel image and Western blot image using the SpotMap software. Images were imported, and the total protein image was aligned on the membrane to the total protein image on the gel. The Western blot image was aligned to the total protein image on the gel by copying the alignment vectors. Spots were detected and assigned as either present or absent.

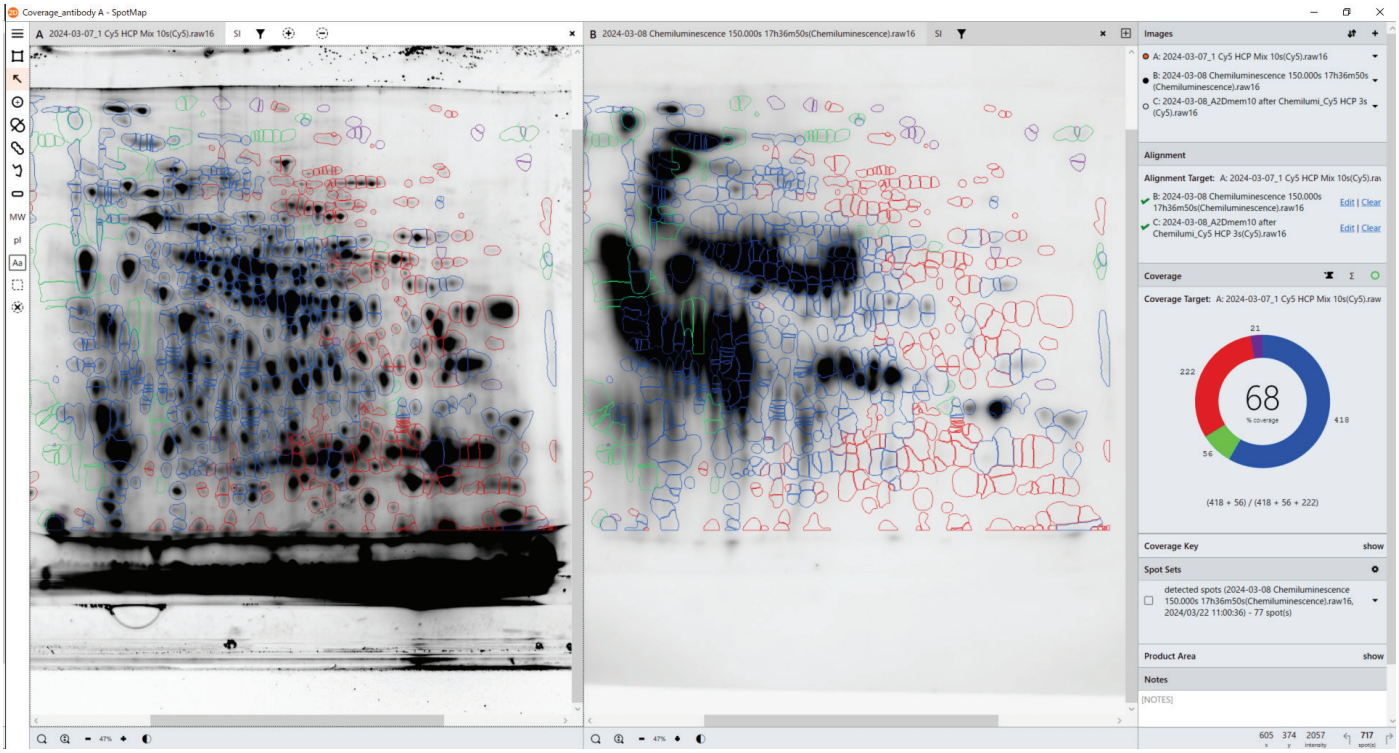

Figure 6: HCP coverage test with Anti-HCP antibody A. Total protein data obtained from the gel (pre-labelling, left), Western blot data with Anti-HCP antibody A (right). SpotMap version number: 5.1.003.

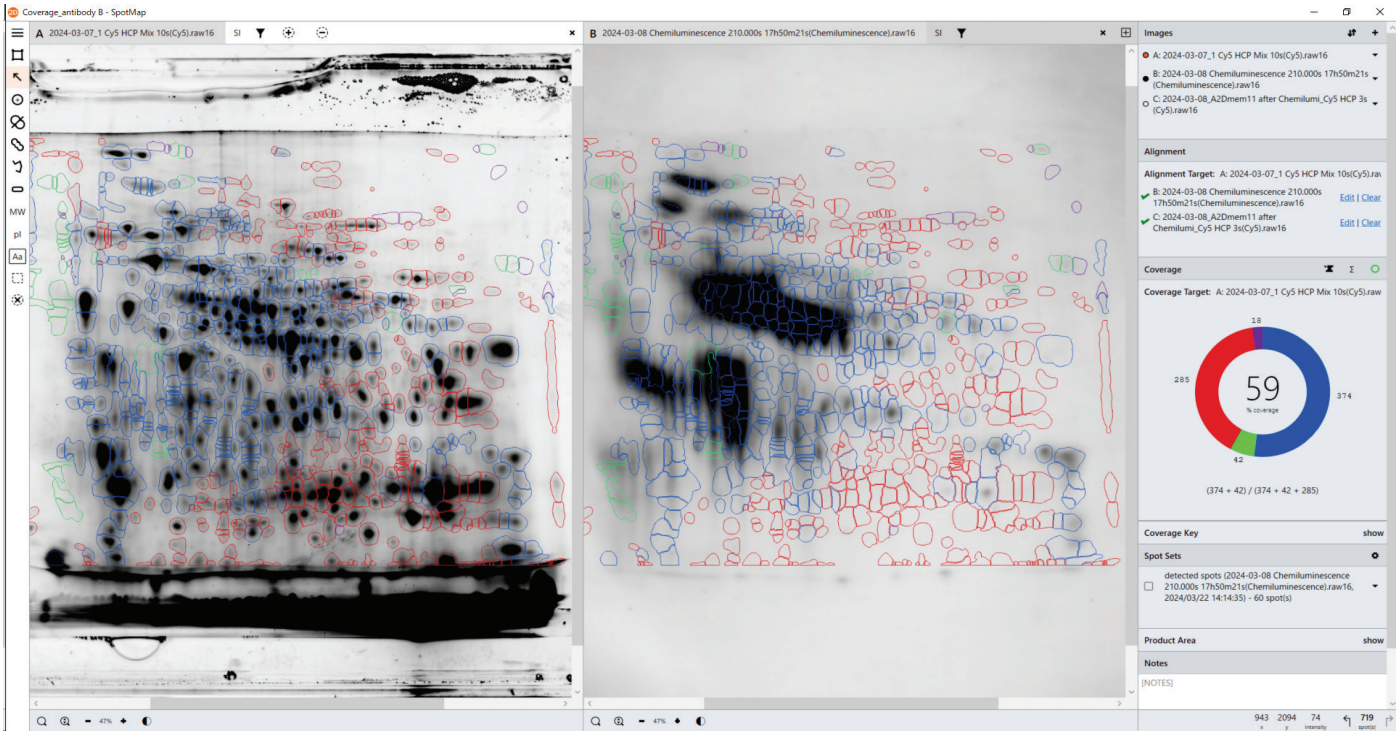

Figure 7: HCP coverage test with Anti-HCP antibody B. Total protein data obtained from the gel (pre-labelling, left), Western blot data with Anti-HCP antibody B (right). SpotMap version number: 5.1.003.

Compared with conventional methods, the required parameter setting adjustments were minimal and it was easy to obtain a reliable coverage percent result due to the high reproducibility of samples run on the Auto2D® device. The anti-HCP antibody coverage score was obtained in as little as 1 hour vs. 12 hours using other 2D analysis software.

TotalLab Software enhances 2-DE and downstream sample analyses

The TotalLab SameSpots software automatically detects spots with high reproducibility on Auto2D® gels with no parameter settings required in advance. By combining the Auto2D® device and SameSpots software, most of the workflow steps can be automated except for image capture. The TotalLab SpotMap software can efficiently detect spots on Auto2D® gels and transfer membrane as shown in the data above, which is suitable for HCP coverage tests.

To learn more about the Auto2D® 2-D Electrophoresis Device, please visit: **SigmaAldrich.com/auto2d**

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