Melanie™ coverage 9.2 software

IMAGE ANALYSIS SOFTWARE

During development and manufacturing of biopharmaceuticals, host cell protein (HCP) impurities must be carefully identified and monitored to guarantee patient safety and drug efficacy. Melanie™ Coverage puts a powerful tool in the hands of scientists developing or utilizing immunoassays such as enzyme-linked immunosorbent assays (ELISA) to monitor HCP impurities in biopharmaceutical products.

The software is focused on the analysis of coverage — the percentage of immunodetection that an antibody reagent offers for the total population of HCPs (Fig 1). It does this by comparing a 2D gel or blot image of proteins detected with an anti-HCP antibody (i.e., secondary image) against an image revealing all HCP antigen proteins (i.e., primary image).

Melanie™ coverage will help you to:

- 3D assisted alignment of spots and automatic detection of all proteins, including those detected only by the anti-HCP antibody
- Improve productivity by visualizing the robustness of your assay with the coverage range and decide to continue the analysis or to perform further optimization
- Gauge the robustness of your analysis, save time, and avoid subjectivity of spot status editing using uncertain spots, which is used to calculate coverage range
- Observe variations in immunodetection at the single protein level—between replicate blots, different antibody reagents, or various antigens
- Provide data export in PDF format to support regulatory reporting

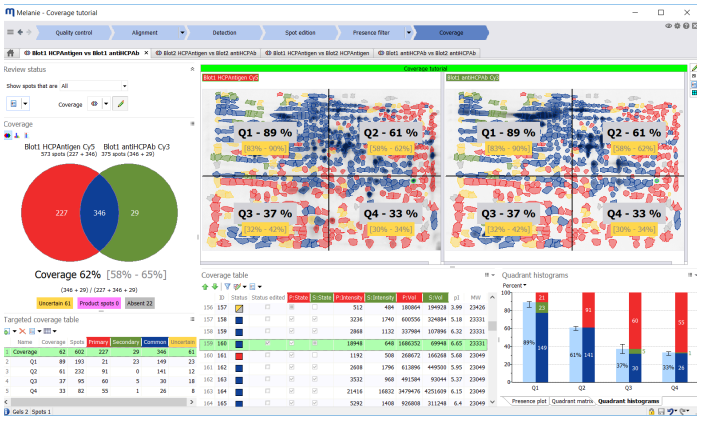

Fig 1. Melanie™ Coverage 9.2 software allows analysis and monitoring of HCP contaminants in biopharmaceutical products from 2D-PAGE gels and blots.

Melanie™ coverage supports all 2D-PAGE based practices:

- Conventional 2DE followed by 2D Western blotting
- 2D differential in blot electrophoresis (2D-DIBE™)
- Immunoaffinity chromatography followed by 2D differential in gel electrophoresis (2D-DIGE)

All common detection agents can be used, including fluorescence, chemiluminescence, and colorimetric and functional group-specific stains.

Melanie™ coverage is the latest addition to the comprehensive Melanie™ software solution for the visualization, matching, detection, quantitation, and analysis of 2D gel electrophoresis (2DE) and Western blot images. Trusted for more than 30 yr by researchers in academia and industry, Melanie™ software is constantly maintained and improved by the SIB Swiss Institute of Bioinformatics, in collaboration with GeneBio and Cytiva.

Streamlined step-by-step workflow

Melanie™ coverage guides you through the image analysis process with a step-by-step workflow, offering functionality and information to simplify and streamline each task. Following the guided steps and checks will consistently yield high-quality results.

1. Quality control

Melanie™ coverage automatically verifies image quality and consistency and provides the necessary feedback to optimize your image capture procedures. Potential issues are highlighted with information for possible solutions. This quality control step saves time that would otherwise be required to analyze images with only limited potential to deliver relevant results.

You can define isoelectric point (pI) and molecular weight (MW) markers so that Melanie™ can calculate theoretical pI and/or MW values for all spots. New functionality in Melanie™ 9.2 further simplifies pI calibration by using predefined or custom templates for immobilized pH gradient (IPG) strips. This increases accuracy of estimated pI data, especially for nonlinear IPG strips (Fig 2).

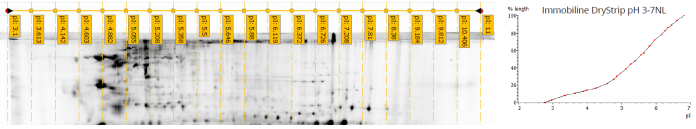

Fig 2. pI calibration with a predefined Immobiline™ DryStrip pH 3–7 NL.

2. Alignment

Alignment—removing the positional variation inherent to electrophoresis—is the most critical step in coverage analysis. When juxtaposing images from different physical objects, alignment is essential, as improper alignment could lead to misinterpretation of the data. For experiments that use DIBE™ or DIGE, intra-gel or intra-blot alignment is not needed (although it can be activated to correct for dye-shifts that can occur in low molecular weight regions).

Alignment is accomplished by finding spot matches between an image in the experiment and its reference image, and then warping the image so that its spots precisely superimpose with those of the reference image. While the Melanie™ software matching algorithm automatically pairs corresponding spots, manual match editing may be necessary when spot patterns are very dissimilar. This often occurs when comparing total HCPs on a gel with immunodetected proteins on an independent blot. Alignment editing views and tools let you work in the way that is most efficient for you:

- Select your preferred display option during alignment (side-by-side, dual color, blink)
- View warped or original images
- Display a grid to visualize deformations in aligned gels
- Edit matches in 3D during alignment review

3D editing allows extremely precise positioning of match vectors, for exceptional alignment accuracy (Fig 3).

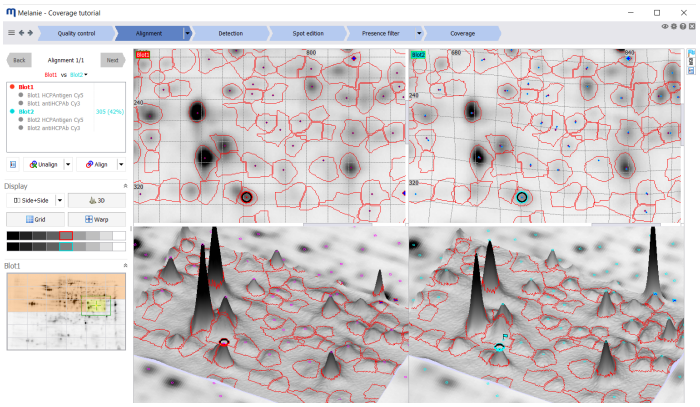

Fig 3. The versatile viewing options in the alignment step enable precise match vector editing.

3. Detection

Once all images are aligned, Melanie™ coverage detects spots on a composite image and propagates the spot boundaries to all images in the experiment. As a result, you will have virtually identical spot patterns on every gel (Fig 4) and 100% spot matching, simplifying subsequent review of spot presence status. Either only HCP-antigen image(s) are used to determine the single spot map, or the anti-HCP antibody image(s) are included as well.

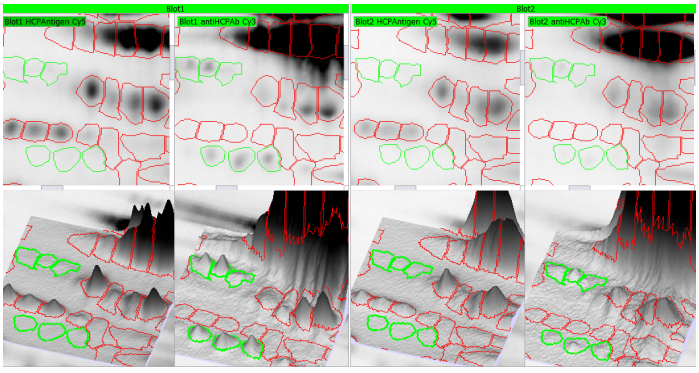

Fig 4. Melanie™ coverage generates identical spot patterns for all images and ensures 100% matching throughout the data set.

4. Spot edition

You can remove irrelevant spots from further analysis by selecting them either manually or based on advanced filter criteria and then excluding them. Where required, you can add spots and split, merge, grow, shrink, or move spots. These spot edits are performed on all images simultaneously, maintaining 100% spot matching throughout the data set and preserving data integrity.

5. Presence filter

Categorization of spots as absent or present is based on a spot-abundance threshold. While it may be easy to fix a value below which you consider a spot as absent, and there likely is a limit above which you confidently regard spots as present, there often is a gray zone. Between the two thresholds, you may want to treat some spots as absent and others as present, depending on factors such as shape, size, and position. These could indicate that the spot is an artifact, that its abundance is overestimated due to overlap with a neighboring spot, or that its abundance is underestimated due to saturation.

- Melanie™ coverage provides for two user-determined thresholds to categorize spots as absent, present, or uncertain (Fig 5). Only the uncertain spots will require manual review, resulting in significant time savings
- The two thresholds can be set to the same value to tag spots as absent or present, without any uncertain spots
- Filter thresholds may be set for each individual image to accommodate variation in dynamic ranges of the images

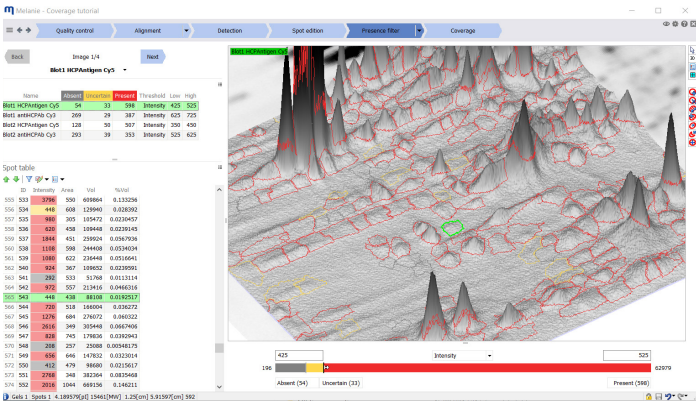

Fig 5. For each image, a slider can be used to set filter thresholds to categorize spots as absent (gray), present (red), and uncertain (yellow).

6. Coverage

For each image pair composed of a primary image (e.g., HCP antigen) and a secondary image (e.g., anti-HCP antibody), Melanie™ coverage determines the coverage status of every spot based on whether the spots in the two images were tagged as absent, present, or uncertain after filtering. Each spot pair will have one of the following coverage statuses:

- Absent: absent from both images (gray)
- Common: present on both images (blue)
- Primary: only present on primary image (red)
- Secondary: only present on secondary image (green)
- Uncertain: uncertain on one or both images (yellow)

Coverage for an image pair can be visualized using a Venn diagram, spot count, or histogram. The diagrams are interactive: clicking in the colored areas (e.g., Common) will select the corresponding spots on the images and in the coverage table.

To obtain a definitive number for the percentage coverage, uncertain spot pairs must be reviewed and assigned a final coverage status (absent, common, primary, or secondary). This process is streamlined with the ability to inspect the spots in 3D and define their status with a single click in the images or table (Fig 6).

Coverage range

To avoid the subjectivity of spot status editing, integrate the notion of uncertainty in your results or simply save time, you can report the coverage range instead of the coverage. The coverage range encompasses the values that the coverage could take, assuming you only edit the spots with uncertain state (Fig 6).

Fig 6. As long as there are uncertain spots, the coverage range is shown in brackets behind the coverage percentage. It is updated, together with the coverage, when uncertain spots are qualified.

This range helps you to improve your productivity by gauging the robustness of your coverage assay so you can decide whether method optimization is preferable before carrying out a detailed spot review.

- If the coverage range is narrow (e.g., 5% to 10%) you can confidently address and edit all uncertain spots
- If the coverage range is broad (e.g., 20% to 30%), method optimization should be considered

Quadrant analysis

In addition to the default coverage for all spots, the software can calculate coverage for given targets, i.e., subsets of spots. For instance, to evaluate the performance of your ELISA antibody reagent to recognize different charge and size categories of proteins — notably the least immunogenic low molecular weight HCPs — you can create coverage quadrants by specifying pI and MW thresholds. You will instantly see in which pI and MW ranges the antibody coverage is suboptimal and where further optimization of immunization strategies is needed (Fig 7).

Since coverage range is assessed for individual quadrants, you will know where to best start reviewing uncertain spots to quickly refine your coverage percentage; just select the quadrant with the largest coverage range. In Figure 7 for example, reviewing the uncertain spots in quadrant Q3 will potentially have most impact on percent coverage.

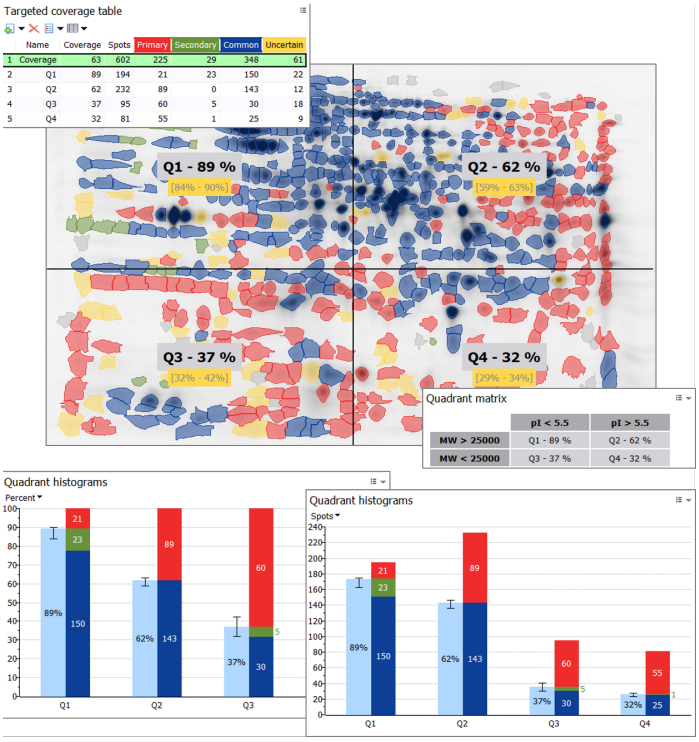

Fig 7. Quadrant analysis of an anti-*E. coli* antibody using 2D-DIBE™ technology. The feature lets you display the name, the coverage percentage and the coverage range (displayed as a black vertical interval in the quadrant histograms) for each quadrant, on the images and in various related reports.

Visual coverage summaries

Coverage analysis is typically carried out on replicate gels and/or blots to estimate the reproducibility of the experiments. Melanie™ coverage allows you not only to analyze multiple HCP antigen and anti-HCP antibody images as part of the same experiment, but also to visually summarize all their coverage information (Fig 8).

Furthermore, your analysis can extend beyond the default HCP-antigen/anti-HCP antibody image pairs. Coverage can be calculated for any desired image pair, even from two separate blots. And as a different coverage formula can be used for each of the three coverage types (primary/secondary, primary/primary, secondary/secondary), you can instantly measure the similarity between two primary images or two secondary images with the Jaccard similarity coefficient (Fig 8). In addition, you can show the coverage summary either for the total coverage, including all spots, or for just one of the quadrants (Fig 8).

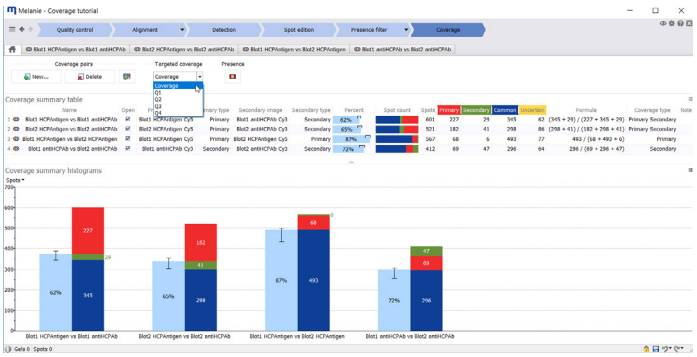

Fig 8. For any desired image pair, coverage information can be included in the customizable summary table and histograms.

Spot presence review across all images

You may have run replicate blots to check how reproducible they are. Maybe you want to compare immunoreactivity of various antibody reagents against the same antigen, or the same antibody against different antigens. For this kind of analysis, it becomes instrumental to go beyond a simple coverage percentage and to zoom in on the presence of individual proteins across all images, to look at and understand variations in immunodetection.

The presence summary helps you with that (Fig 9). It summarizes the absence/presence of each spot on every image in the experiment to let you:

- Review uncertain spots and edit their presence state while visualizing the spot on all images
- Identify spots for which the presence state was edited
- Verify if modifications of spot presence state are congruent with the presence filter thresholds using the presence plot. The latter shows where the abundance value of a spot is situated relative to the presence filter thresholds and therefore whether your edits seem reasonable
- Select proteins that were detected with a certain frequency in specific image subsets (e.g. all, primary, or secondary images)
- Select proteins with specific expression profiles. This enables you to compare how and where different anti-HCP antibodies vary in terms of HCP recognition or to tell what proteins are similar or different between HCP antigens

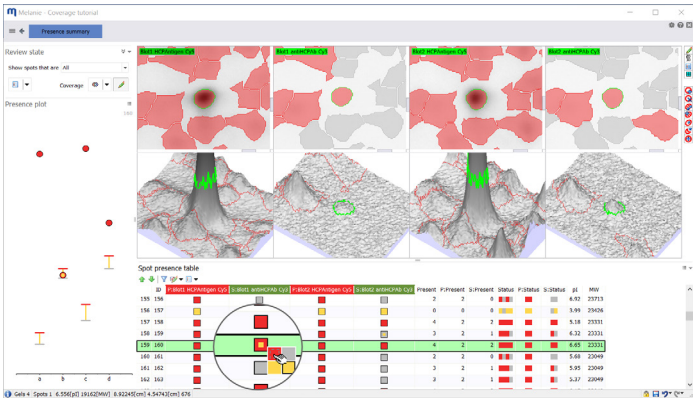

Fig 9. The presence summary lets you visually review every spot across all images, for a detailed understanding of differences in immunodetection.

Documentation and compliance support

While generic and process-specific immunoassays are critical components of HCP contaminant detection, regulatory agencies require demonstration that the polyclonal antibody mixture used in the assay is broadly reactive against a wide range of potential HCPs. Melanie™ coverage supports all current standard practices for determining coverage of HCP by generic or specific antibodies.

To submit a dossier to health authorities such as FDA and EMA or archive all essential project information, you can automatically generate a comprehensive PDF report that includes quality control checks, workflow parameters used for the analysis, results, images, and all tables and plots.

Free viewer functionality

With Melanie™ coverage installed, you can view your gel images and check their quality even without a license. Any collaborator will be able to view the results of an analysis carried out with the licensed software, so you can easily share your work and scientific discoveries. Installing the license will unlock all functionality, such as aligning, detecting, and analyzing results.

Integration

Support the collaborative efforts of researchers with seamless sharing of project data within a network and import/export features that allow users to send analyzed results (including images, spots, matches, annotations, spot sets, and spot presence) to external partners.

Additional features enable the seamless integration of Melanie™ software into your laboratory workflow:

- Compatibility with Amersham™ HCP DIBE™ CHO coverage kit from Cytiva, CyDye™ DIGE Fluor minimal dyes, saturation dyes from the CyDye DIGE™ Fluor Labeling Kit for Scarce Samples, and Immobiline DryStrip™ gels
- Direct analysis of image files acquired with Amersham™ Typhoon™ scanners, Typhoon™ FLA scanners, and Amersham™ Imager 600 and 680
- Spot data export in PDF, text, Excel®, and XML format for further downstream analysis
- Clipboard support to copy gel images, graphics, and data tables to other programs
- Annotation capabilities that allow gel objects to be linked to external search engines or databases

Specifications

PC requirements

Operating system	Windows® 7, 8 or 10 operating systems. 64-bit versions are recommended for maximum performance
Administrative privileges	To install Melanie™ coverage, the license server, and the license
RAM	Minimum 4 GB. Increased memory enhances the performance when many and/or large images are analyzed
Video card	Capable of 24-bit color. The video card driver needs to support OpenGL™ (v1.2 or later)–ensure that the latest compatible driver is installed.
Color resolution	Minimum 24-bit color
Screen resolution	Minimum 1024 × 768 pixels
Web browser	A browser is required to view the software documentation and access databases on the web. Recommended browsers are: Google Chrome™17+, Mozilla™ Firefox™10+, and Internet Explorer® 11+.

Input file specifications

File format	TIFF, GEL, MEL, IMG, GSC, or 1SC grayscale images. Importing DIGE gels from DS files allows fully automatic gel naming and grouping
Resolution	For normal sized gels (ca. 20 × 20 cm), a resolution between 150 and 300 dpi (169 to 85 µm) is optimal. For mini gels (approx. 7 × 7 cm), with smaller spots, a higher resolution (e.g., 600 dpi or 42 µm) is indicated. As a rule of thumb, resulting images should be at least 1000 × 1000 pixels, and at most 2500 × 2500 pixels, with smallest spots having a diameter of at least 5 to 10 pixels
Bit depth	12-bit minimum, 16-bit recommended
File names	For DIGE gels, it is recommended that the file names for the group of two or three images contain a common string and their respective dye names (Cy™2, Cy™3, Cy™5)

Ordering information

Melanie™ coverage can be purchased as a stand-alone application, or as an upgrade to Melanie™ Classic or DIGE software. When purchased as a stand-alone, it can be upgraded with the Melanie™ Classic and DIGE™ modules.

Product	Product code
Melanie™ 9 coverage Perpetual Node-locked license[1]	29705440
Melanie™ 9 coverage Perpetual Floating license[2]	29705442
Melanie™ 9 coverage Perpetual Site Floating	29705324
Melanie™ 9 Package[3] Perpetual Node-locked	29705340
Melanie™ 9 Package[3] Perpetual Floating	29705331
Melanie™ 9 Package[3] Perpetual Site Floating	29705325

After 2 yr of warranty period	Product code
Melanie™ 9 coverage Renewal[4] Node-locked	29705439
Melanie™ 9 coverage Renewal[4] Floating	29705441
Melanie™ 9 coverage, Renewal[4]/Upgrade Site Floating	29705323
Melanie™ 9 Package, Renewal[4]/Upgrade Site Floating	29705326

Other Melanie™ modules	Product code
Melanie™ 9 Classic Perpetual Node-locked license	29705335
Melanie™ 9 Classic Perpetual Floating license	29705337
Melanie™ 9 DIGE™ Perpetual Node-locked license	29705338
Melanie™ 9 DIGE™ Perpetual Floating license	29705336

Related products	Product code
Typhoon™ 5	29187191
Typhoon™ RGB	29187193
ImageQuant™ 800 Fluor	29399484

[1] A node-locked license is locked to a specific computer. Software will only be able to run in fully functional mode on that computer.

[2] A floating license is available anywhere (floating) on a network, instead of being tied to specific computer. It is locked to a license server host that runs the license manager daemon. Software will be able to run in fully functional mode on any computer that can access the identified license server. The license is "counted," meaning that the license manager keeps track of the number of "seats" hosted by that server.

[3] Package software contains Classic, DIGE, and Coverage module.

[4] A Renewal license is available after the 2 yr warranty for access to the latest updated software version.

cytiva.com/imagingsoftware

For local office contact information, visit cytiva.com/contact

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