Melanie™

classic and DIGE 9.2 software

IMAGING SYSTEMS, SOFTWARE, AND ACCESSORIES

Differential Protein Expression Analysis

Melanie™ 9 is a comprehensive software solution for the visualization, matching, detection, quantitation, and analysis of 2D gel electrophoresis (2DE) and Western blot images. The Melanie™ Classic and DIGE modules are designed to detect statistically significant differences in protein expression between experimental groups, with high objectivity, sensitivity, and confidence.

- **Melanie™ Classic** is suitable for analysis of conventional 2DE gels.
- **Melanie™ DIGE** additionally supports analysis of 2D Differential in Gel Electrophoresis (2D-DIGE) experiments and other multiplexed technologies without internal standard.

Designed for ease of use and efficiency, the Melanie™ application places the experimental design at the center of the analysis and makes 3D imaging available throughout the analysis workflow.

Melanie™ 9 software replaces the DeCyder™ 2D and ImageMaster™ 2D Platinum applications. It was developed with a single goal in mind: help you draw more reliable conclusions from all your 2D electrophoresis data. Trusted for more than 30 years by researchers in academia and industry, Melanie™ is constantly improved and maintained by a team at the SIB Swiss Institute of Bioinformatics, in collaboration with GeneBio and Cytiva.

Key benefits

- Melanie™ 9 lets you detect real differences in protein expression with high objectivity, sensitivity, and confidence. This capability is made possible through image quality control, 3D view assisted image alignment, 100% spot matching, and the use of statistical tests suited to the design of your experiment.
- With fewer false positives, save time and money otherwise wasted on downstream analysis of protein changes that are merely due to biological variation.
- Reduce analysis time with the intuitive step-by-step workflow and strategies that increase efficiency of alignment without requiring spot editing.
- Clear image analysis guidance and robust default settings help new users get started quickly.

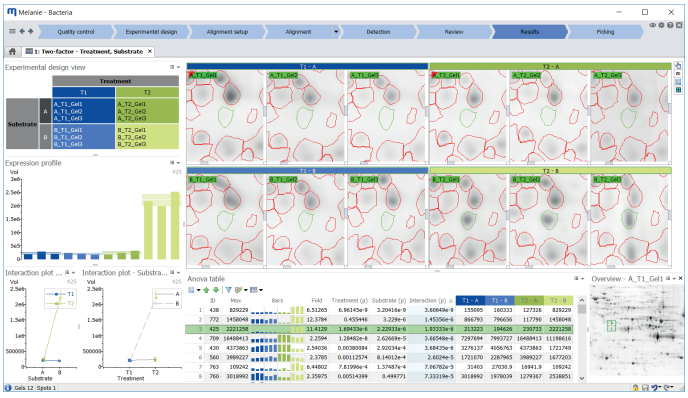

Fig 1. Melanie™ 9 allows easy analysis of two-factor experiments.

- Stay in control of your analysis. Melanie™ 9 adapts to your specific needs by offering a high degree of flexibility at all levels, from image display choices, through normalization options and advanced workflow settings.
- The free viewer functionality enables you to easily share your work and scientific discoveries, facilitating collaboration with colleagues wherever they are located.
- Melanie™ functionality can be extended with the Coverage module to allow HCP (host cell DNA) antibody coverage analysis. You can perform all your gel-based protein expression profiling with one user-friendly, comprehensive application.

Feature highlights

- Step-by-step workflow for easy guidance through the analysis.
- Image quality control to optimize image capture.
- Experimental design wizard to easily define the experimental design variables.
- Alignment strategies that increase efficiency and minimize match editing work.
- 100% spot matching and virtually identical spot patterns for all images, for high data confidence.
- Advanced normalization options to extend the range of applications.
- Specific statistical support for many one- and two-factor analyses, improving detection of true differences (Fig 1).
- Automatic presentation of spot statistics.

Support for a wide range of applications

Melanie™ supports common detection agents, including fluorescence, colorimetric, and functional group-specific stains.

The software fully exploits the advantages of DIGE and other multiplex gel electrophoresis techniques. These advantages include:

- separation and co-migration of more than one sample per gel,
- using size- and charge-matched dyes to label the different samples, and
- the ability to incorporate an internal standard on every gel (Fig 2).

By providing an inherent link between samples, the internal standard simplifies gel-to-gel matching and allows normalization of protein quantities across samples.

The advanced normalization options enable analysis of protein abundance in experiments where it cannot be assumed that the distribution of expression levels is similar between samples. These options can be applied, for example, to host cells expressing recombinant protein, or to analysis of samples from different subcellular fractions. This capability opens up new applications, from expression analysis to process development for monoclonal antibodies.

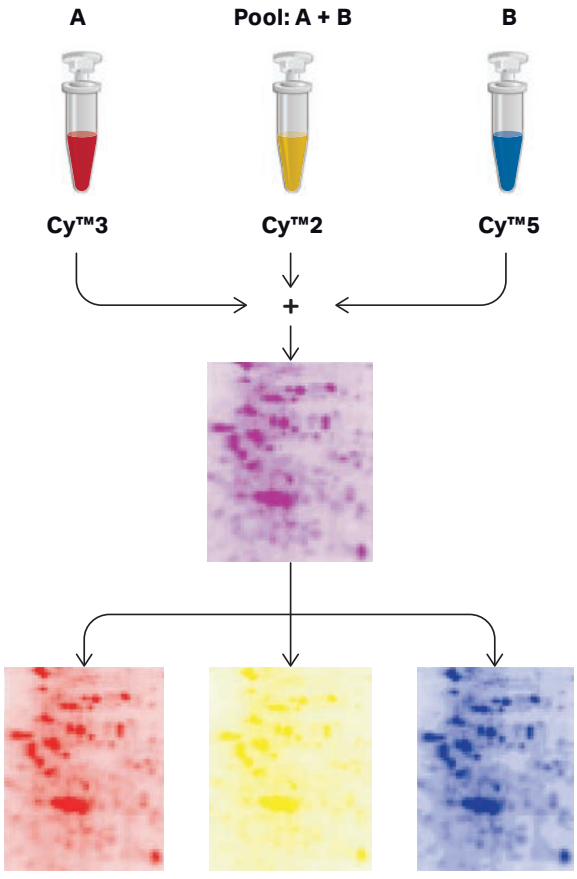

Fig 2. Overview of 2D-DIGE (three-dye example).

Streamlined step-by-step workflow

A step-by-step workflow guides you through the image analysis process, offering the functionality and information needed for the current task. The workflow lays out the steps and checks to deliver the highest quality results.

1. **Quality control:** Import, visualize and verify the quality of your images and their consistency within the data set. If required, re-scan gels or edit images (crop, flip, rotate, scale, invert). Then validate the images you want to take forward for further analysis. You can carry out pI/MW calibration by defining a few pI and MW markers in the dedicated interface. Melanie™ will then calculate theoretical pI and/or MW values for all spots.
2. **Experimental design:** Use the experimental design wizard to create one of the common designs, define factors and factor levels, and assign images to the different treatments. Specify additional variables you might want to investigate, and ensure you have a consistent and balanced experimental design.
3. **Alignment setup:** Optimize alignment efficiency by aligning images first within groups of similar images. The different groups will be matched by aligning their respective reference images. You can group images based on factors defined in the experimental design or build your own group hierarchy.
4. **Alignment:** Align the images in your alignment hierarchy to remove positional variation between gels. Review each alignment pair using the dedicated tools, and edit matches where necessary.
5. **Detection:** Fine-tune the detection parameters and choose the images that will be used to generate a representative spot pattern.
6. **Review:** Check the spot pattern, edit spots or make corrections in the alignment if needed. Select irrelevant spots either manually or based on advanced filter criteria, and exclude them from further analysis. Then review the normalization before continuing with the statistical analysis.
7. **Results:** Identify spots of interest with the dedicated tools and statistical tests that are automatically adapted to your experimental design. Validate spots using spot filters, advanced annotations, various plots, and versatile viewing options.
8. **Picking:** Choose spots for picking and export them for further downstream analysis.

Image quality control

Automatic image quality control functionality provides feedback to optimize your image capture procedures (Fig 3), and verifies that all images in the data set have consistent characteristics such as size or intensity encoding. Potential issues are highlighted and information provided on how to solve them. This quality control step helps eliminate images with only limited potential to deliver relevant results.

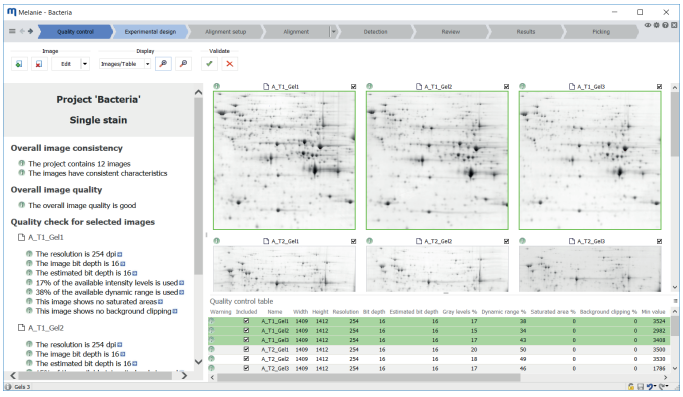

Fig 3. Quality control step in the workflow

Experimental design centered

The proper design of an experiment is critical to obtaining relevant results from any 2D gel electrophoresis study. We believe you should be able to fully exploit your experimental design information at all stages of the analysis. The Melanie™ experimental design wizard helps you describe common one- and two-factor designs and seamlessly assign images to the different factor levels (Fig 4).

The experimental structure is exploited at all stages of the analysis workflow. It is used to propose specific alignment strategies, image display layouts, and appropriate statistical tests and data analysis tools.

Fig 4. The two steps in the experimental design wizard.

Efficient alignment strategies

Alignment, the most critical and time-consuming step in the analysis, removes the positional variation inherent to the electrophoresis process. Alignment finds spot matches between each image in the experiment and its reference image, and then warps every image so it precisely superimposes with the reference image.

You can increase alignment efficiency and minimize match editing work by defining an appropriate alignment strategy. By aligning images in a hierarchical manner, rather than selecting a single image as a reference, you can reduce the number of tedious alignments that are typical for data sets with many dissimilar gels.

For DIGE experiments, intra-gel alignment is not needed. However, it can be activated to correct for dye-shifts that can occur in low molecular weight regions.

100% matching

Multiple user-friendly alignment options, including 3D editin, allow extremely precise positioning of match vectors, for exceptional alignment accuracy.

- Select your preferred display option during alignment editing (side-by-side, dual color, blink).
- View the warped or original images, or display a grid to visualize deformations in aligned gels.
- Edit matches in 3D during alignment review (Fig. 5).

Once all images are aligned, the software detects spots on a fusion 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 6) and 100% spot matching, without missing values in your statistical analysis. This process leads to highly reproducible, objective results that you can report with confidence.

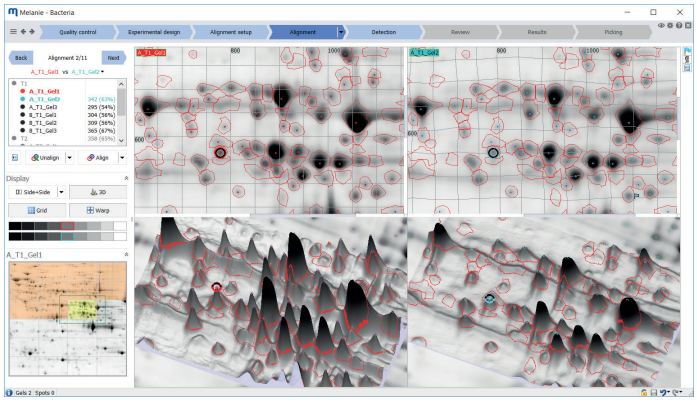

Fig 5. Extensive viewing options in the Alignment step enable precise match vector editing.

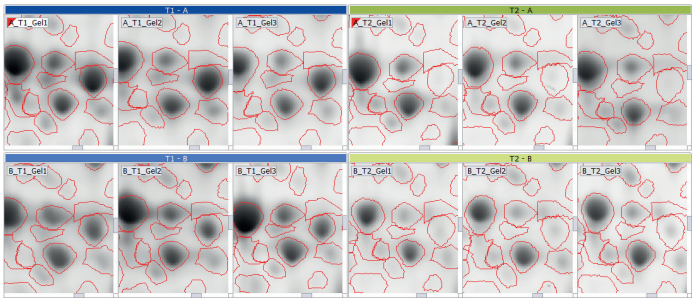

Fig 6. Melanie™ 9 generates identical spot patterns for all images and ensures 100% matching throughout the data set.

Advanced normalization options

The spot abundances used in Melanie™ 9 can be normalized using one of the available normalization functions.

The default ratiometric normalization method, as well as the alternative total volume normalization method work on the assumption that the majority of all proteins in a gel maintain their overall expression level between the various samples in an experiment. This approach can be difficult to apply to samples that have only a few protein spots, or to samples where the majority of the proteins differ in expression between samples. Therefore, Melanie™ 9 offers spike normalization as an alternative normalization method. This option normalizes data to spike proteins, selected to have a minimum of interference/overlay with other protein spots. Data can be normalized to proteins that are either added to samples or are already present as housekeeping proteins known to have a constant concentration.

In some experiments where spike normalization is not possible or practical, it may not be appropriate to normalize all images to a single reference. For instance, to study the effect of a treatment on the protein expression in very different subcellular fractions, it may be more appropriate to normalize only within samples of the same fraction. This method provides accurate quantitative abundance measurements for the treatment effect within each fraction, even if you may only draw qualitative conclusions from comparisons between fractions. Melanie™ 9 enables this capability by letting you specify groups within which you want to normalize.

One- and two-factor statistical analyses

Melanie™ 9 offers specific statistical support for the most common one- and two-factor experimental designs, including designs that incorporate a subject or blocking factor. A carefully designed experiment can still be wasted if subsequent statistical analysis does not take into account the structure of the data. By applying the appropriate model for the Analysis of Variance (ANOVA) available in Melanie™ 9, your ability to detect true differences will improve considerably.

The default results screen automatically displays the statistics that are most applicable to your experimental design. However, you can create and manage additional analyses.

Figure 7 shows an application example of a two-factor DIGE experiment analyzed with Melanie™ 9.

For experiments that have been designed to study three or more primary factors, or that integrate more advanced design notions and are therefore not specifically supported, you can still align and detect your images, filter, edit and normalize spots, and use some of the statistical tools for exploration. You can then export your data for appropriate statistical analysis with third party software, under the guidance of a statistician.

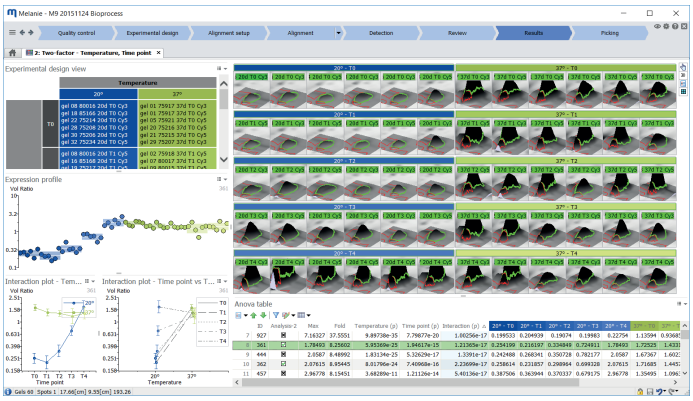

Fig 7. Example of a two-factor DIGE experiment analyzed with Melanie™ 9. This bioprocess optimization study looked at the protein expression in bacteria cultivated at two different temperatures (20°C, in blue, and 37°C, in green), at 5 different time points (T0, T1, T2, T3, T4, from top to bottom). For each treatment, 6 replicates were prepared, thus generating a total of 60 different samples run on 30 gels. A pooled internal standard was included as a third sample on each gel. As can be seen in the interaction plots (bottom left) and expression profile (middle left), when cultivated at 20°C, the bacteria need significantly more time to produce the selected protein in the same quantities as in the 37°C culture.

Versatile analytical methods

Typical questions for 2D image analysis are:

- Do any proteins or protein patterns characterize a specific biological state (e.g., tumor versus normal tissue)?
- Could any identified proteins be used for the development of diagnostic markers?

The various analytical methods in Melanie™ 9 can be used to answer these questions and select proteins for picking, digestion, and subsequent analysis by mass spectrometry. Selection of protein spots can be based on criteria such as statistical significance of change, magnitude of change, spot abundance, or any combination of criteria.

The available analytical methods are:

- Statistical tests, to perform differential expression analysis. Depending on your design, one-way or two-way ANOVA will be applied. The statistical significance of change can be used to reduce the data set to only those proteins that show a defined change in expression level.
- Principal component analysis (PCA) to identify outliers in the data and check whether gel images cluster according to the experimental design groupings.
- Expression profiles and interaction plots, to visualize the protein abundances and variability in different sample groups.
- Descriptive statistics such as the mean, standard deviation, and coefficient of variation, to summarize the magnitude and variability of the spot values within a population. Fold change can be used to compare expression levels between different populations.
- Scatter plots, to analyze relationships between different spot quantities or statistical measures, in particular to examine gel similarities or experimental variations.

Flexible, user-friendly interface

With the Melanie™ interface, adjusting your visualization options is both easy and flexible. You can control how you want to group and lay out your images for viewing, and you can choose your preferred combination of 2D and/or 3D views, which can both be used for match and spot editing.

The software offers fully dynamic 2D and 3D displays, tables, expression profiles, and plots, in which both content and selection are continuously updated and synchronized. By selecting a spot in one view, information on the same spot is displayed in the other views.

Free viewer functionality

With Melanie™ 9 installed, even without a license, you can view your gel images, check their quality and consistency, verify if you have a consistent and balanced experimental design, and plan your alignment strategy. 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.

Seamless integration

To support the collaborative efforts of researchers, Melanie™ 9 ensures seamless sharing of project data within a network and provides import/export features that allow users to send analyzed results (including images, spots, matches, annotations, and spot sets) to external partners.

Many additional features enable the seamless integration of our software into your laboratory workflow:

- Compatibility with Cytiva CyDye™ DIGE Fluor minimal dyes and saturation dyes from the CyDye™ DIGE Fluor Labeling Kit for Scarce Samples.
- Direct analysis of image files acquired with Cytiva Amersham brand Typhoon™ scanners, Typhoon™ FLA scanners, and Amersham™ Imager 600 and 680.
- Fully automated integration with spot-picking robots. Before exporting the pick file, you can carry out pI/MW calibration to help interpretation of mass spectroscopy-based identification data. This process is as simple as defining a few pI and MW markers in the dedicated interface.
- Spot data export in text, Excel®, and XML formats 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™ 9, 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 (169–85 µm) is optimal. For mini gels (ca. 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

Product	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
Melanie™ 9 DIGE upgrade from Classic Node-locked license	29705333
Melanie™ 9 DIGE upgrade from Classic Floating license	29705341
Melanie™ 9 Classic Renewal Node-locked	29705334
Melanie™ 9 Classic Renewal Floating	29705342
Melanie™ 9 DIGE Renewal Node-locked	29705339
Melanie™ 9 DIGE Renewal Floating	29705332

Related products	Product code
Melanie™ 9 Coverage Perpetual Node-locked license	29705440
Melanie™ 9 Coverage Perpetual Floating	29705442
Melanie™ 9 Coverage Perpetual Site Floating	29705324
Melanie™ 9 Package Perpetual Node-locked	29705340
Melanie™ 9 Package Perpetual Floating	29705331
Melanie™ 9 Package Perpetual Site Floating	29705325
Typhoon™ 5	29187191
Typhoon™ RGB	29187193
ImageQuant 800 Fluor	29399484

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