Melanie™ 8.0 software

Melanie is a comprehensive software solution for the visualization, matching, detection, quantitation, and analysis of 2-D gel electrophoresis images. It is used to establish the statistical significance of protein expression changes between experimental groups. The software is suitable for a wide range of applications and methods, including conventional 2-DE and 2-D DIGE (difference gel electrophoresis) experiments, Western blots, and other multiplexed technologies.

Melanie 8.0 has been developed to supersede the DeCyder™ 2D and ImageMaster™ 2D Platinum applications. It is a complete revamp of the previous software, with a single goal in mind: help you draw more reliable conclusions from all your 2-D electrophoresis data, with ease.

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 GE Healthcare. This access to talent, expertise, and technology, in combination with direct feedback from users and specialists in the field, supports development of highly relevant solutions for your gel-based protein expression profiling.

Key benefits

- Melanie lets you detect real differences in protein expression with high objectivity, sensitivity, and confidence. This capability is made possible through image quality control, 100% spot matching, and the use of statistical tests that take into account the design of your experiment.
- With fewer false positives, you save time and money otherwise wasted on downstream analysis of protein changes that are merely due to biological variation.
- Your analysis time will be substantially reduced with the intuitive step-by-step workflow and powerful strategies that increase efficiency of alignment without requiring spot editing.
- If you are a new user, clear image analysis guidance and robust default settings will help you to get started quickly.

Main features

- 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 strategy to 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 and 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 include an internal standard on every gel (Fig 2). This internal standard is ideally created by pooling aliquots of all biological samples in the experiment. When an internal standard is used, the default spot protein abundance is expressed as Volume ratio, comparing the spot volume on an image with the corresponding spot volume of the internal standard. Using the internal pooled standard approach simplifies gel-to-gel matching, removes system variability, and delivers the most accurate quantitation possible.

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.

Guided step-by-step workflow
Melanie guides you through the image analysis process with a step-by-step workflow, offering the functionality and information needed for the current task. With the control and validation tools dedicated to each analysis stage, you can feel confident that you will accomplish the necessary steps and checks for the highest quality results.

1. **Quality control**: 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.

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 experimental 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. Then specify the reference images and reference groups for alignment.

4. **Alignment**: Align the images in your alignment hierarchy to remove positional variation between gels. Systematically review each alignment pair using the dedicated tools, and edit matches where necessary.

5. **Detection**: Fine-tune the automatically calculated detection parameters and choose the images that will be used to generate a representative spot pattern. Spots will be detected and quantified on all images within seconds.

6. **Review**: Check the spot pattern, editing spots or making corrections in the alignment if needed. Select spots using the advanced criteria in the spot filter, to include or 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.

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**Fig 2. Overview of 2-D DIGE (three-dye example).**
Image quality control

Melanie automatically controls the quality of your images so that you get the necessary feedback to optimize your image capture procedures (Fig 3). It also checks 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 will save the time that would otherwise be required to analyze images with only limited potential to deliver relevant results.

Experimental design centered

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

Efficient alignment strategies

Alignment is the most critical and time consuming step in the analysis. It aims to remove the positional variation inherent to the electrophoresis process. Alignment is done by finding spot matches between each image in the experiment and its reference image, and then warping every image so it precisely superimposes with the reference image.

Melanie allows you to increase alignment efficiency and minimize match editing work by defining an appropriate alignment strategy. Melanie’s alignment strategies avoid aligning all images to a single reference that may be quite dissimilar to many gels in the data set and would therefore generate an unnecessarily large number of difficult alignments.

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

You can select your preferred display option during alignment editing (side-by-side, dual color, blink). You can choose whether you want to look at the warped or original images, or display a grid to visualize deformations in aligned gels. But Melanie goes further by also allowing you to view your images in 3-D during alignment review. This feature is extremely useful as it shows the exact correspondence of spots and therefore allows more rigorous positioning of match vectors (Fig 5).

The experimental design structure is subsequently used throughout the analysis workflow to propose specific alignment strategies, image display layouts, and appropriate statistical tests and data analysis tools.

Fig 3. Quality control step in the workflow.

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

Fig 5. The elaborate viewing options in the Alignment step enable precise match vector editing.
Once all images are aligned, Melanie 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 6. Melanie 8 generates identical spot patterns for all images and ensures 100% matching throughout the data set.

Advanced normalization options

The spot abundances used in Melanie (i.e., the Volume for non-DIGE data sets; and the Volume ratio, standardized to the within-gel internal standard, for DIGE data sets) can be normalized using one of the available normalization functions.

The default Ratiometric normalization, as well as the alternative Total volume normalization, 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 samples where the majority of the proteins differ in expression between samples. Therefore, Melanie 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 enables this capability by letting you specify groups within which you want to normalize.

One- and two-factor statistical analyses

Melanie offers specific statistical support for the most common one- and two-factor experimental designs, including designs that comprise a subject or blocking factor. This feature is important, because 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), your ability to detect true differences will improve considerably.

The default results screen automatically displays the spot statistics for the test that is 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 8.

Fig 7. Example of a two-factor DIGE experiment analyzed with Melanie 8.

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.

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.
Versatile analytical methods

Typical questions for 2-D 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 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 methods are:

- Statistical tests, to perform differential expression analysis. Depending on your design, Student's t-test, one-way ANOVA, 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.
- 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. The 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 Melanie, 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 2-D and/or 3-D views.

The software offers fully dynamic 2-D and 3-D 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

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. This way, you will be up and running in no time once you get your purchased license. Installing the license will unlock all functionality, such as aligning, detecting, and analyzing results.

Another benefit is that any collaborator that has Melanie installed, even without license, 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.

Seamless integration

To support the collaborative efforts of researchers like you, Melanie 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 GE's 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 GE's Amersham™ Typhoon™ scanners, Typhoon FLA scanners, and Amersham Imager 600.
- Spot data export in Text, Excel®, and XML format for further downstream analysis.
- Fully automated integration with spot-picking robots. Before exporting the pick file, you can carry out pI/MW calibration to help interpretation of mass spectrometry-based identification data. This process is as simple as annotating a few known protein standards with their pI and MW values.
- 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

<table>
<thead>
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<th>Requirement</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Operating System</td>
<td>Windows® 7, 8 or 10 operating systems. 64 bit versions are recommended for maximum performance</td>
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<tr>
<td>Administrative privileges</td>
<td>To install Melanie, the license server, and the license</td>
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<tr>
<td>RAM</td>
<td>Minimum 2 GB. Increased memory enhances the performance when many and/or large images are analyzed</td>
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<tr>
<td>Video card</td>
<td>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</td>
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<tr>
<td>Color resolution</td>
<td>Minimum 24-bit color</td>
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<td>Screen resolution</td>
<td>Minimum 1024 × 768 pixels</td>
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<tr>
<td>Web browser</td>
<td>A browser is required to view the software documentation, print reports, and access databases on the web. Recommended browsers are: Google Chrome™17+, Mozilla™ Firefox™10+, and Internet Explorer® 11+</td>
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### Input file specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Requirement</th>
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<tr>
<td>File format</td>
<td>TIFF, GEL, MEL, IMG, GSC, or 1SC grayscale images. Importing DIGE gels from DS files allows fully automatic gel naming and grouping</td>
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<tr>
<td>Resolution</td>
<td>For normal sized gels (ca. 20 × 20 cm), a resolution between 150 and 300 dpi (169–85 micron) is optimal. For mini gels (ca. 7 × 7 cm), with smaller spots, a higher resolution (e.g., 600 dpi or 42 micron) 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</td>
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<tr>
<td>Bit depth</td>
<td>12-bit minimum, 16-bit recommended</td>
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<tr>
<td>File names</td>
<td>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 (Cy2, Cy3, Cy5)</td>
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### Ordering information

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<td>Melanie Classic</td>
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