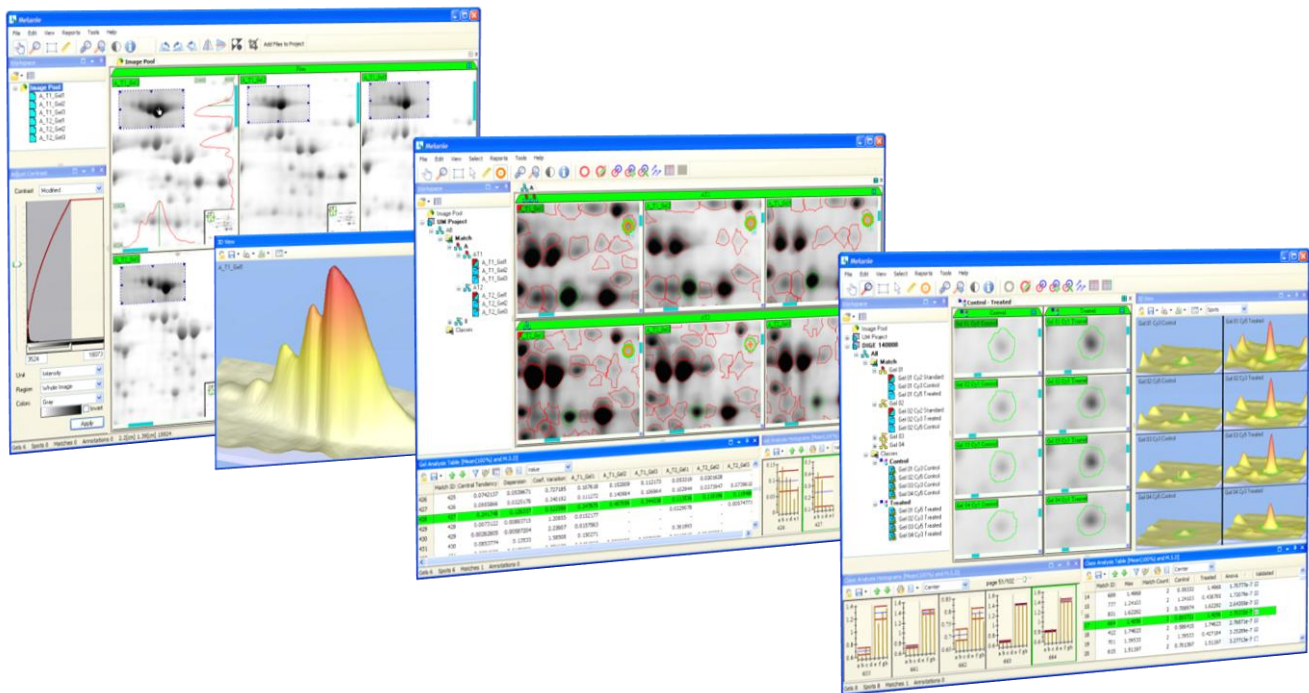


melanie 7.0

2D gel analysis made easy and fast



Melanie 7.0 provides a flexible interface to visualize, explore and analyze 2D electrophoresis gel images, in order to identify protein markers of interest through differential expression analysis. The solution can be applied to a wide range of 2D gel and blot experiments, including DIGE.

CONVENIENCE AND SPEED

Melanie simplifies and improves analyses of 2D gels and identification of protein markers of interest.

Many of the functions found in earlier versions have been enhanced and simplified, and significant new features have been added for greater convenience and speed. The user interface has been improved and new interaction modes simplify even the most complex analysis.

Want to know more or try a fully functional demo version?
Then visit www.2d-gel-analysis.com

MELANIE 7.0 OFFERS

- Well established methods for all 2D experiments, including single stain and DIGE experiments
- Easy import and image viewer functionality
- Effective spot detection and matching solutions
- Versatile analytical methods
- User-friendly and flexible interface
- Adaptable visualization tools
- Seamless integration in your laboratory workflow
- Quality and data integrity

ESSENTIAL CAPABILITIES

WELL ESTABLISHED METHODS

Melanie is the fruit of more than one decade of collaborations with eminent partners and users worldwide. The application has attained a high level of maturity over the years and has evolved into a solution that can be applied to a wide range of 2D gel and blot experiments. The software has comprehensive tools that are well-suited to the traditional gel analysis workflow as well as to the most challenging data sets (Fig 1).

Melanie is also designed for DIGE applications, which allow the separation and co-migration of more than one sample per gel, using size- and charge-matched CyDye™ DIGE Fluor dyes to label the different samples, and the inclusion of an internal standard for every spot on every gel. It uses the patented co-detection algorithm of the DeCyder™ 2D Differential Analysis Software (DeCyder 2D) to fully exploit the advantages offered by multiplexing.

A unique analysis pathway is used for all your gel studies, both for conventional 2D electrophoresis and DIGE gels. The three steps in the workflow are:

- *Import & Control* – Import images and view, explore, edit and calibrate them before further analysis.
- *Organize & Process* – Allow gel comparisons by detecting and matching images.
- *Analyze & Review* – Define biological groups, perform statistical analysis and review the results.

In the workspace, you can control and verify the status of a gel analysis, and have direct access to the desired step in the workflow.

BENEFITS

- Flexible, user-friendly interface
- Supports all 2D gel image formats including GEL, MEL, IMG, GSC, ISC, and TIFF
- Compatible with many electrophoresis products
- Import/export in Text, Excel™ and XML format
- Powerful Undo/Redo
- Annotation capabilities that allow gel objects to be linked to external search engines or databases
- pI and MW calibration
- Excellent customer support backed by committed Melanie team

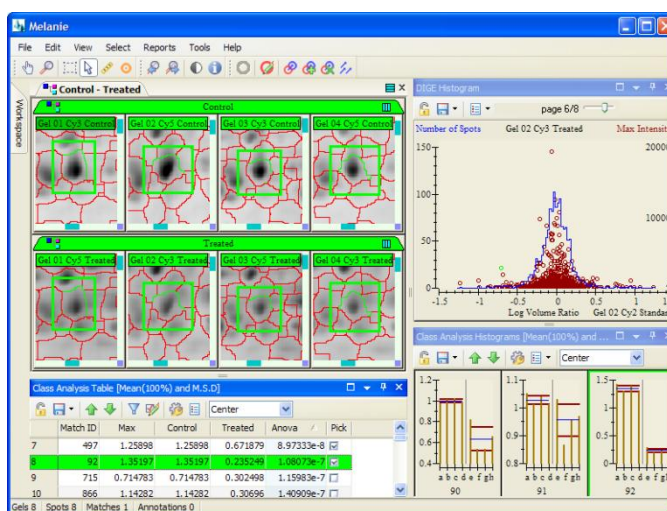


Fig 1. Melanie 7.0 can be used for a wide range of 2D gel experiments, including DIGE.

EASY IMPORT AND VISUALIZATION

Melanie automatically recognizes all 2D gel image formats during import, including TIFF images and *.GEL, *.MEL, *.IMG, *.GSC, and *.ISC format files. For DIGE experiments, Melanie furthermore detects probable combinations of co-run images that can be validated with a single click.

The software therefore provides excellent image viewer functionality, without limiting the number of images that can be displayed simultaneously.

Before performing analysis, the images can be edited (rotated, flipped, cropped, and inverted) or calibrated to remove image scanning variations. The contrast settings and color palettes can also be adjusted at any time.

The desired gels can subsequently be inserted into a project, by simple drag and drop, for further analysis.

EFFECTIVE SPOT DETECTION AND MATCHING SOLUTIONS

The cornerstones of a fast and successful 2D gel analysis are efficient spot detection, accurate spot quantitation, and robust gel matching. In Melanie, these functionalities integrate effective solutions to avoid or minimize editing of the results.

SPOT DETECTION ALGORITHMS OPTIMIZED FOR DIGE AND NON-DIGE APPLICATIONS

- For *conventional 2D images*, the Melanie algorithm provides only a few easily adjustable spot detection parameters, for distinguishing real spots from noise.
- For *DIGE images*, the powerful co-detection algorithm from DeCyder 2D simultaneously processes one, two, or three images derived from a single gel.

MINIMAL SPOT EDITING

The advantage of the individual spot detection for conventional 2D images is that the image data from every single gel is fully exploited. However, spot detection differences can occur. In particular, some spots are split in different ways among gels to be compared.

In addition to simplified spot editing tools, version 7.0 provides robust solutions to deal with detection variations between gels without calling for spot editing:

- By exploiting the capability to define multiple matches, you can create "composite spots" that are treated as unique entities in the quantitation.
- Spots can be propagated from one image to the other matched images. This allows for quantification of identical areas on all gels.

HIERARCHICAL POPULATION MATCHING

All images in an experiment are not equally easy to compare, even when the gels are run in a highly controlled way. Typically, gels belonging to the same biological group are easier to match than images from different biological populations. This information is not exploited when matching all images against a unique arbitrary reference image and often results in missed spots in the analysis.

By combining Melanie's powerful parameter-free matching algorithm with hierarchical population matching, more efficient match designs can be created. Figure 2 shows an example of a match hierarchy, where images are first matched within a match set, and two of these match sets are then compared at a higher level.

The advantages of hierarchical population matching are substantial:

- By first matching gels within biological populations, one minimizes the number of difficult match combinations. This significantly reduces time spent on match editing.
- With one or two easily created landmarks (tie points), the entire experiment is matched in a matter of seconds, and matches are automatically propagated at each level of the unrestrained match hierarchy.
- A new strategy for comparing populations reduces the number of spots missed in the analysis, by minimizing the influence of the global match reference.
- Any protein spot can be matched against equivalent spots in other images, without the need to copy spots to the reference.

ANALYTICAL METHODS

- *Scatter plots, to analyze gel similarities or experimental variations.*
- *Descriptive statistics of central tendency and dispersion, to summarize the magnitude and variability of the spot values within a population.*
- *Factor analysis, to help identify underlying sources of variation and indicate whether, and how well, spot groups and populations might be separated.*
- *Statistical tests, to perform differential expression analysis. The statistical significance of change can be used to reduce the data set to only those proteins that show changes in expression level. The tests include one-way ANalysis of VAriance (ANOVA), Mann-Whitney/Wilcoxon, and Kolmogorov-Smirnov.*
- *Overlapping measures (summarize each gel population by an interval and compute the overlap between these intervals), to identify significant protein expression variations without making any restrictive assumptions, as do the various statistical tests.*
- *Histograms, to visualize expression profiles.*

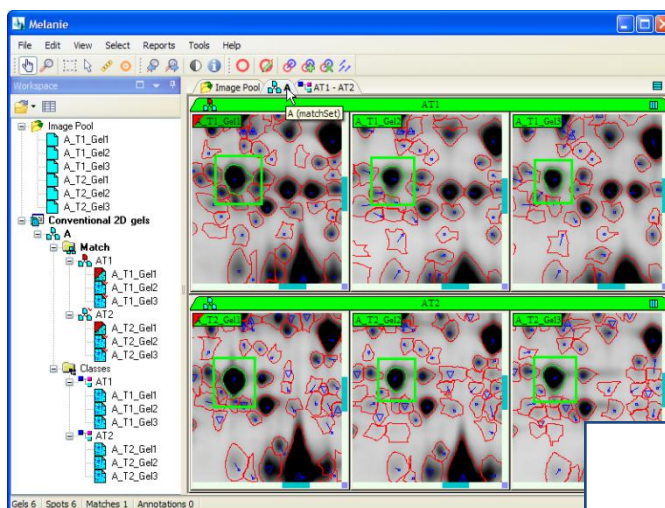
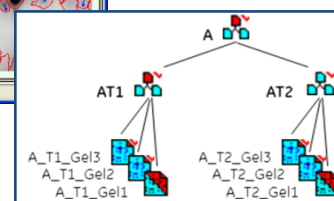


Fig 2. Scheme of a match hierarchy A, with sub match sets AT1 and AT2, and its display in the workspace and MatchSet sheet.



VERSATILE ANALYTICAL METHODS

Typical questions for 2D image analysis are:

- Are there proteins or protein patterns that might be characteristic of a biological state (e.g. tumor versus normal tissue)?
- Are there proteins that might be used for the development of noninvasive tests (i.e. diagnostic markers)?
- How many classes exist in a given data set?

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 multiple criteria such as statistical significance of change, magnitude of change, spot volume, or any combination of criteria.

FOCUS ON YOUR PROBLEM

Melanie has a full set of dedicated tools to focus the analysis on your specific problem.

Based on the quantitative and qualitative filters in the reports, protein spots that are not of interest can be disabled or tagged in order to concentrate the analysis on a specific subset of spots. Such spot sets are saved for later retrieval or combination.

In Melanie, further focus is achieved by continuously synchronizing the current selection in all the different views, to allow a clear overview of all critical information (Fig 3).

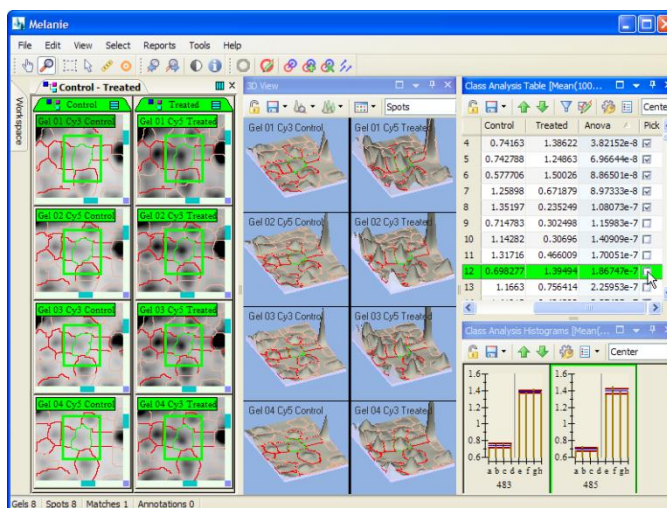


Fig 3. All views in the interface are linked to allow a clear overview of the critical information. Pink spots have been temporarily disabled so that they do not appear in the reports.

USER-FRIENDLY INTERFACE WITH FLEXIBILITY

Whether working with 10, 50, or 500 images, Melanie allows users to display, manipulate, and process gel data with unmatched flexibility and ease.

The application layout and gel images can be freely reorganized to optimize space and visibility in accordance with personal preferences. The intelligent multi-worksheet display guarantees a structured and consistent presentation, where related images always remain grouped and can be instantly viewed.

Melanie 7.0 offers fully dynamic tables, histograms, plots, and 3-D views in which both content and selection are continuously updated to stay up-to-date with the corresponding sheet that contains the gel images.

Each user can customize his or her personal work environment, for instance by creating custom toolbars, menus or report templates.

ADAPTABLE VISUALIZATION TOOLS

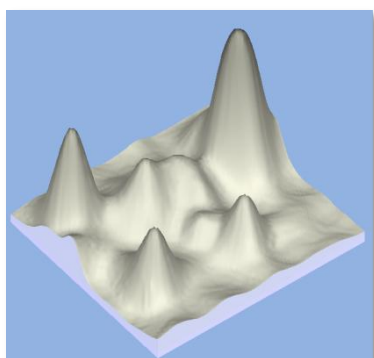
Melanie offers numerous features to visualize different aspects of gel images (Fig 4).

The various modes to move and zoom the images allow the user to become familiar with the spot patterns or to navigate to the desired location in the image, possibly aided by the Overview feature.

Signal intensity can be inspected using the Adjust Contrast feature, the new 3-D View, or the Profile curves.

The software further provides specific tools to visually compare different images:

- Automatically displayed match vectors allow easy verification of match results, while superimposing spots can be viewed in a different color.



NEW AND IMPROVED FEATURES

- Simplified import and visualization of images
- Simplified project management with easier population set up.
- Dedicated landmark tool
- Higher match efficiency with hierarchical match designs
- Management of multiple matches and composite spots.
- All spots matched with the user selection are automatically highlighted
- Tables, graphs, plots and 3D views are continuously synchronized with the selection on the images
- One-click to choose desired layout of sheets and panes
- Option to filter out spots from the analysis
- Student t-test replaced with ANOVA, expressed as probabilities
- Adaptive display of histograms
- Revamped 3D View
- Reviewed contrast adjustment feature
- Reorganized menu structure with icons
- Custom and context-related toolbars
- Single tool to select/edit spots and annotations
- Measure tool to compute pixel, pI, MW, or linear (centimeter or inch) distances between spots
- And much more...

- Automatic warping aligns gel images, eliminating differences in spot position. Aligned images are then overlaid to produce dual channel images with clearly visible differences in protein expression.
- Synchronized and simultaneous 3-D views of multiple gels can be displayed.

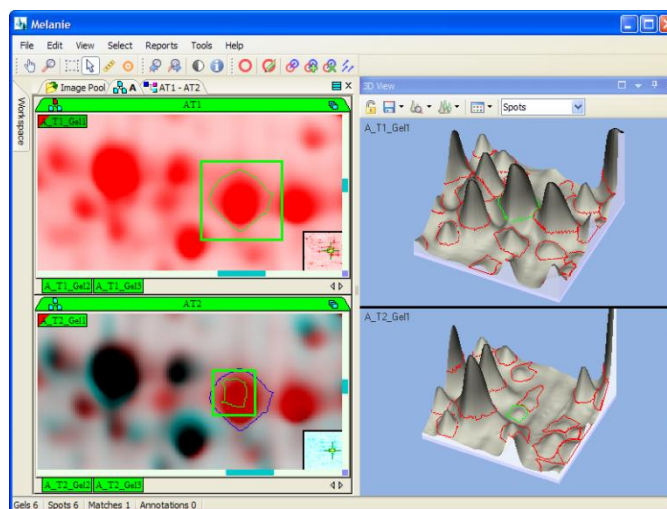


Fig 4. Different images can be compared by displaying synchronized 3D views and dual color images (red spots correspond to those from the sheet reference A_T1_Gel1). The Overview at the bottom right of each gel image shows the location of the currently visible area.

Grids can be used to visualize deformations in aligned gels, or to evaluate distances between spots, in terms of pixel coordinates, pI/MW units, or linear measures (cm or inch). The pI/MW calibration grids use pI and MW values of known standards to automatically calculate values for all other spots and propagate these through all matched images.

SEAMLESS INTEGRATION

To support the collaborative efforts of researchers, Melanie 7.0 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, spot sets, etc.) to external partners.

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

- Support of multiple recommended image formats, TIFF images, GEL, MEL, IMG, GSC, and 1SC files.
- Direct image acquisition from Twain-compatible scanners.
- Export spot data in Text, Excel™, and XML format for further downstream analysis.
- Fully automated integration with spot-picking robots.
- 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 data bases.