

- 1
- 2
- 3
- 4

# Imaging and Analysis

Tools for Acquisition and Analysis  
of Protein Expression Data





Expression proteomics compares patterns of proteins expressed in different samples. Several complementary tools are available for analysis of protein expression, including the following three technologies: sample preparation, two-dimensional (2-D) electrophoresis, and imaging and analysis. Stained proteins separated by 2-D gel electrophoresis are imaged and analyzed to compare the quantitative and qualitative protein expression patterns of different samples. Proteins of interest can be recovered from the gels for mass spectrometric identification, and the identifications captured by PDQuest™ 2-D analysis software.

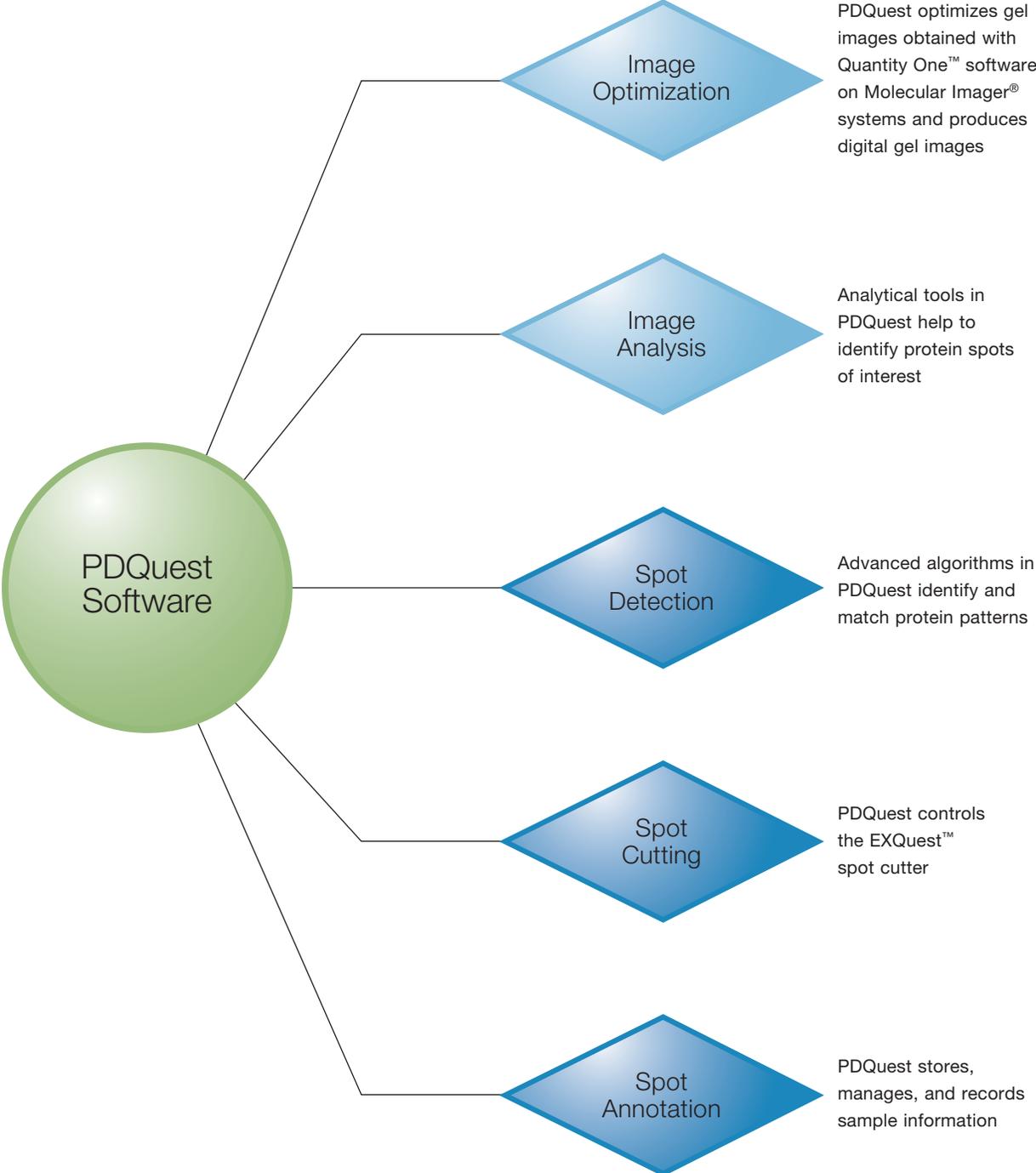
### Imaging and Analysis

Bio-Rad provides a wide assortment of imaging systems to meet various 2-D gel imaging requirements. Sophisticated image analysis software simplifies the analysis of 2-D data and makes the process more efficient by automating spot detection and analysis. PDQuest software controls the imaging systems, collects and manages large amounts of image data, and rapidly and reliably analyzes the information. 2-D gel analysis remains the most effective way to evaluate protein content changes under different conditions, and is the foundation for understanding protein expression in complex biological systems. PDQuest is the tool that helps you make sense of the complexity that is present in any expression proteomics experiment. Bio-Rad's integrated imaging and analysis systems simplify the entire analysis process:

- Image acquisition and optimization
- Image analysis
- Spot detection
- Spot cutting for protein identification
- Spot annotation

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Integration



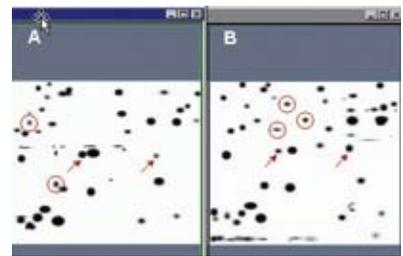
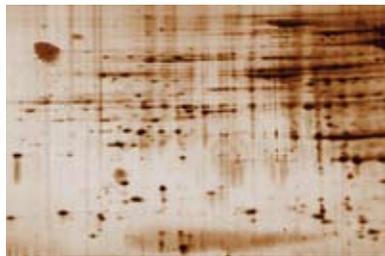
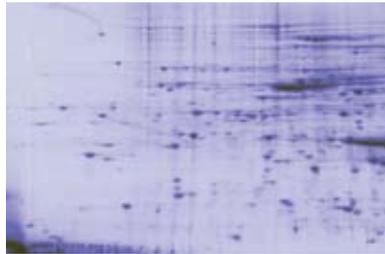
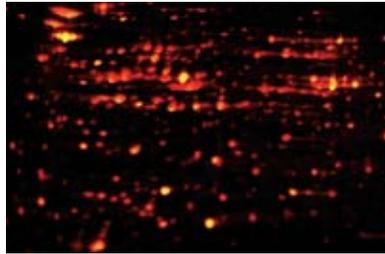
## Analysis Workflow

The 2-D gel process is an effective way to prepare samples for subsequent analysis of expressed protein changes under different biological conditions. Bio-Rad's expression proteomics system allows you to:

- Analyze the quantitative differences in proteins between conditions
- Separate and isolate the isoforms of a protein created by posttranslational modifications
- Create a static parallel display of the proteins in a sample
- Recover specific proteins of interest in a state that is compatible with mass spectrometric identification

PDQuest software drives the process of analysis for expression proteomics and facilitates the entire analysis workflow:

- Image
- Compare
- Analyze
- Excise
- Identify



### Image

Bio-Rad's selection of Molecular Imager systems gives you the flexibility to use the protein detection approach best suited for your analysis needs. Choose from instruments with fluorescence, colorimetric, radioisotope, or chemiluminescence detection capabilities. PDQuest software is integrated seamlessly with Quantity One image acquisition software, which controls all Molecular Imager systems.

### Compare

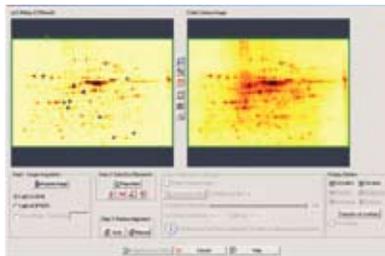
PDQuest software compares two or more gel images, revealing changes in protein expression in response to different treatments, growth conditions, or disease states.



## Analyze

PDQuest offers superior performance for analysis and databasing of 2-D gels. You can reveal subtle differences among gels using its sophisticated analysis tools, which include:

- Measurement of quantitative and qualitative differences in protein expression
- Dynamic range of 5 orders of magnitude for precise quantitation
- Identification of statistically significant differences among expressed proteins
- Higher-level Boolean analysis
- Multiple report formats and review tools



## Excise

PDQuest ensures integration of the multiple workflow steps essential for successful expression proteomics studies — acquisition of gel images, spot cutting, sample tracking, and annotation.



## Identify

Protein identifications are seamlessly retrieved by PDQuest and used to annotate the gel image. Mass spectrometric results can be used as the basis for searches of public and proprietary databases to identify unknown proteins. PDQuest records the identifications along with other sample and experimental information.

## Image

### Molecular Imager System Selection Guide

Application	Personal Molecular Imager™ (PMI™)	PharosFX™ Plus	VersaDoc™ MP 4000	VersaDoc™ MP 5000	Gel Doc™ XR	ChemiDoc™ XRS	GS-800™ Calibrated Densitometer
<b>Protein Staining, 1-D Gels</b>							
Coomassie Blue	—	2	5	3	4	4	5
Silver stain	—	2	5	3	4	4	5
SYPRO Ruby protein gel stain	—	5	5	3	4	4	—
Flamingo™ fluorescent gel stain	—	5	4	3	4	4	—
<b>Protein Staining, 2-D Gels</b>							
Coomassie Blue	—	2	4	2	3	3	5
Silver stain	—	2	4	2	3	3	5
SYPRO Ruby protein gel stain	—	5	4	3	3	3	—
Flamingo fluorescent gel stain	—	5	4	3	3	3	—
Pro-Q	—	5	4	3	3	3	—
Cy2, Cy3, Cy5	—	5	5	4	—	—	—
<b>Blot Detection</b>							
Coomassie Blue	—	2	5	3	4	4	5
Silver stain	—	2	5	3	4	4	5
SYPRO Ruby** protein blot stain	—	5	5	5	—	—	—
Immun-Star™ (chemiluminescent kits)	—	—	3	5	—	4	—
Chemifluorescence**	—	5	5	5	1	1	—
Qdot**	—	5	5*	5*	1*	1*	—
<b>Isotopic</b>							
Radiolabel detection	5	5	—	—	—	—	—
X-ray film detection	—	3	5	3	4	4	5

— Not recommended; 1–5, recommendation level (5 = highest). \* Custom filter required. \*\* Optimal with low-fluorescence PVDF.

## Image Acquisition

Analysis of high-quality 2-D gels is a basic requirement for investigating changes in protein expression. Bio-Rad's Molecular Imager systems have the flexibility, resolution, and dynamic range you need to capture as much quantitative data as possible, with as much accuracy as needed, regardless of your requirement for a few gels, many gels, or multiplexing samples. Driven by Quantity One software and seamlessly integrated into the data flow of PDQuest software, each Bio-Rad imaging system allows you to capture the full extent of your data and increases your ability to obtain meaningful information from each experiment. Choose from densitometry, fluorescence, chemiluminescence, and phosphor imaging systems. Multispectrum imaging options and easy-to-use application-based software let you visualize spots stained or labeled with any method applicable to 2-D gels.



### Molecular Imager Pharos FX Systems

- Patented fiber-optic scanning and direct laser excitation to minimize optical distortion
- Sample imaging from top to accommodate wide range of samples
- Self-alignment of external lasers before each scan for reliable performance
- User-configured custom emission filters to provide unlimited choice of fluorophores in visible spectrum
- Transillumination screen to perform gel documentation for colorimetric stains
- Image acquisition with Quantity One software and options for FDA 21 CFR Part II
- Ergonomic design with a small footprint



### Molecular Imager VersaDoc MP 4000 System

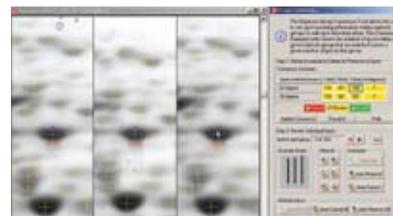
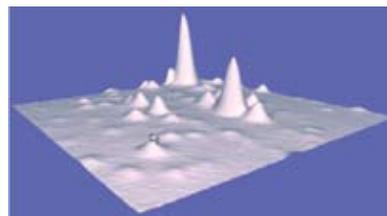
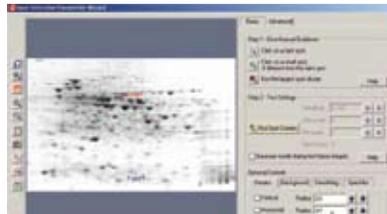
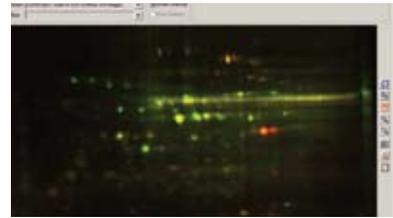
- State-of-the-art high-resolution gel imaging with 3.2 million pixels
- Single- and multicolor fluorescent, chemiluminescent, chemifluorescent, and colorimetric samples
- Cooled CCD technology for great sensitivity and dynamic range
- Pixel binning for greater detection range
- Excellent data uniformity using flat fielding (CV  $\leq$ 5%)
- Automated acquisition
- Easy to customize and upgrade



### Molecular Imager GS-800 Calibrated Densitometer

- Calibrated transmissive and reflective scanning
- 12-bit data and 36.3  $\mu$ m resolution, allowing analysis of closest spots on a gel
- Accurate quantitation of stained gels within a large dynamic range (0–3.0 OD)
- Unique design to accommodate wet samples of variable thickness
- Installation qualification and operational qualification (IQ/OQ) of calibration functions

## Compare and Analyze



### PDQuest 2-D Analysis Software

PDQuest drives the analysis of 2-D gel-based experiments because it uses:

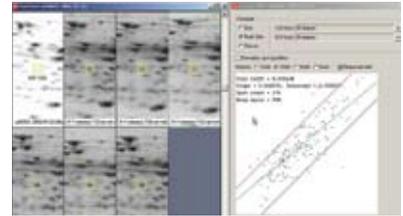
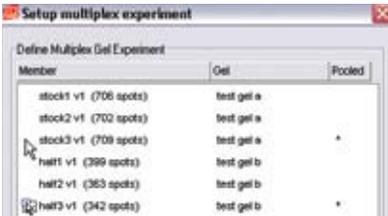
- Automated spot matching algorithms for high-confidence comparisons of protein expression patterns
- Normalization between gel images to provide accurate comparative results
- Statistical and qualitative query reports that allow you to tease out subtle changes in protein expression
- Merging of multiple images of the same gel to give consistent quantitative results

### Spot Detection

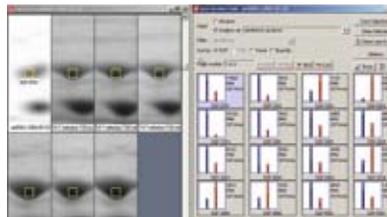
- Use intuitive Spot Detection wizard's sophisticated algorithms to automatically and accurately detect spots on all your gels
- Optimize spot detection parameters based on experimental conditions to ensure reproducibility
- Visualize both raw and filtered data in 2 or 3 dimensions to precisely define your spots

### Matching

- Effortlessly automatch gels in a matter of seconds
- Fine-tune spot detection using the Replicate Group Consensus tool to edit spot criteria and ensure that images precisely match
- Reselect parameters or edit as needed
- Review multiplex dye images or compare multiple images using versatile multichannel viewer
- Easily locate and display sets of spots with defined characteristics



Name	Median Int.	Total Spot Qty.	Total Density	Check-loaded	Comp.
reference 1 20 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 100 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 120 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 24 hrs.	1.0000	0	0.0000	0.0000	0.0000



Name	Median Int.	Total Spot Qty.	Total Density	Check-loaded	Comp.
reference 1 20 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 100 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 120 hrs.	1.0000	0	0.0000	0.0000	0.0000
reference 1 24 hrs.	1.0000	0	0.0000	0.0000	0.0000

## Normalization

- Gels to be compared can be normalized to account for differences in staining intensity, imaging, and sample loading variability
- A variety of normalization options accommodate different experimental conditions
- Multiplexing capability

## Quantitation

- Gels or replicate groups of gels are quantitated
- A spot review tool allows you to ensure that the quantitation corresponds with the spot intensity
- Gaussian spot modeling aids in quantitation of expressed protein spots — even overlapping ones

## Analysis and Reporting

- Proteins from different samples are compared using a scatter plot; each spot on the graph is hyperlinked to a spot on the gels
- Quantitation data are displayed in table format for export to Excel or other software and databases
- PDQuest gives a variety of options for reporting expression changes

## Excise and Identify

### PDQuest Drives Automation With the EXQuest Spot Cutter

Bio-Rad's expression proteomics system is a powerful analysis platform because it provides control of data as well as protein separation.

Studying complex biological relationships requires reproducible methods and tools for purifying, separating, imaging, analyzing, and excising interesting proteins before they can be identified through mass spectroscopy.

The EXQuest spot cutter offers fast, hands-free gel excision with unparalleled accuracy and reliability. Bio-Rad Laboratories has reinvented gel excision with convenience and precision in mind, and has made it accessible to all types of laboratories. Whether you require occasional gel cutting or high-throughput gel cutting on a daily basis, EXQuest will improve your laboratory workflow and increase the accuracy of your results.

PDQuest controls the EXQuest spot cutter based on analysis sets you create.



EXQuest spot cutter

## Automation

### Intelligent Workflow

- Gel excision is integrated with gel analysis software tools, allowing easy transition from gel analysis to gel cutting
- Gel image is matched to the original analysis image on the day of gel cutting, to increase precision by accounting for tearing, shrinking, swelling, or fading of protein spots
- Easily review spots to be cut, with real-time imaging of gel on cutter alongside original analysis image
- A variety of editing tools allow you to create multiple cuts on large spots (automatically or manually), move cuts, select different gels within a set, or pool cuts from multiple gels

### Hands-Free Multi-Gel Imaging and Plate Processing

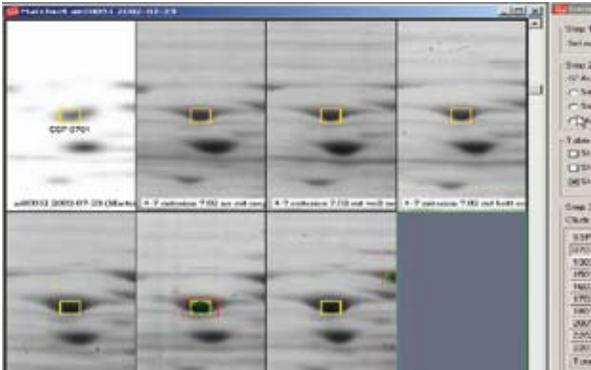
- Imaging and cutting of up to 4 Criterion™ gels at a time
- High-throughput capacity of up to 4 microplates

### Sophisticated Liquid Handling

- Vacuum pickup and liquid-facilitated delivery for the most accurate gel recovery available, >99.5%
- Automatic calculation of dispensing volumes based on gel thickness and well volume
- Automated gel hydration to ensure gel integrity
- Multiple wash/rinse stations with “flow-through” tip rinsing for the most accurate spot identifications

### High-Speed Precision Robotics

- “New-generation” Cartesian robotics for reliable delivery of up to 600 spots per hour
- Resolution of 100 μm for unbeatable precision
- Smooth operation for any benchtop

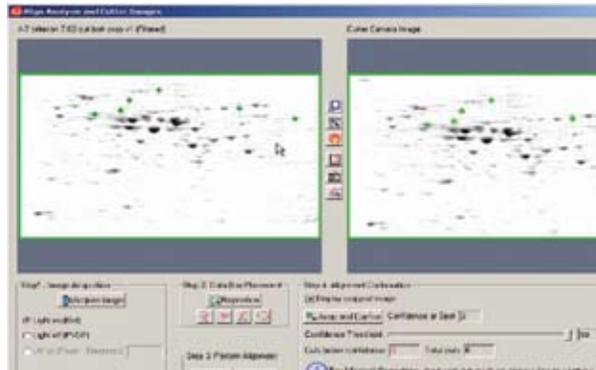


## Spot Cutting

The EXQuest spot cutter is the only spot cutter that can work with all of the following:

- Freestanding 2-D and 1-D gels
- 2-D and 1-D gels cast on plastic backing
- PVDF and nitrocellulose blots
- Coomassie Blue, silver, and other visible stains
- Flamingo, SYPRO Ruby, and other fluorescent stains

Spot cutter targeting matches the spot cutter image to the original analysis image, and makes the cut setup fast and accurate, with minimal user review.



## Annotation

Protein expression studies are powerful because changes in protein patterns can be correlated with many characteristics. The PDQuest annotations tools provide a convenient way to compile all the information about a protein you discover. The preset annotations give you a start, and you can add as many new categories as you want. Annotations are directly associated with the spot number within the experiment (matchset), so all information is directly associated with the gel image, providing a powerful means of comparison and review.

- Annotate images with text, URLs, and files
- Export annotated images to presentations or documents

## Support



### Application Support

Bio-Rad's expression proteomics experts offer field support to customers worldwide. Each specialist has a solid understanding of the technology and research experience that will help you find solutions to your experimental needs.

### Sales Support

Bio-Rad's trained, knowledgeable customer support staff operates worldwide. They can help you choose the best system to fit your particular needs.

For more information, contact your local Bio-Rad sales representative or visit us on the Web at [www.expressionproteomics.com](http://www.expressionproteomics.com)

### Global Technical Support

Bio-Rad has over 30 years of experience in 2-D technology. Our worldwide technical support staff is highly trained and can advise you on how to obtain good results. They can help with troubleshooting or with advice on suitable tools for sample preparation or other expression proteomics technologies.

### Research and Development

Bio-Rad's expression proteomics R&D team develops ideas into reliable research tools. By continuing to make 2-D electrophoresis a more reproducible and robust technology, R&D helps customers to focus on research, rather than perfecting techniques.

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