



# Focal Points

Application Note FP-131



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## 2D Imaging with the UVP BioSpectrum® 800 System

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Two dimensional SDS polyacrylamide gel electrophoresis remains a cornerstone for protein research, giving an array highly resolved proteins by separating a protein sample first by the isoelectric point and second by size (1, 2, 4, 5). The end result is an SDS PAGE gel with 100's to 1000's of "spots", generally circular or ellipsoid in shape with diameters from fractions of a millimeter to a cm or more. The individual spots typically represent just one protein, making 2D SDS PAGE an ideal way to isolate proteins for further analysis. In some cases, the spots will contain more than one protein, but the reduction in complexity by using 2D SDS PAGE greatly simplifies further analysis.

Once separated, the proteins need to be visualized prior to image acquisition and analysis (3, 4, 5). The sample can be fluorescent tagging before separation, with the end result the separated proteins are already fluorescent and ready to be fluorescently imaged with UV or visible excitation light. If the protein is not tagged at the start, then the protein must be stained via Coomassie blue, silver, or SYPRO Ruby fluorescent stains. Coomassie Blue is an easy to use diffusion based stain that leaves the spots blue and straightforward to image using a visible white light table, and is illustrated below.

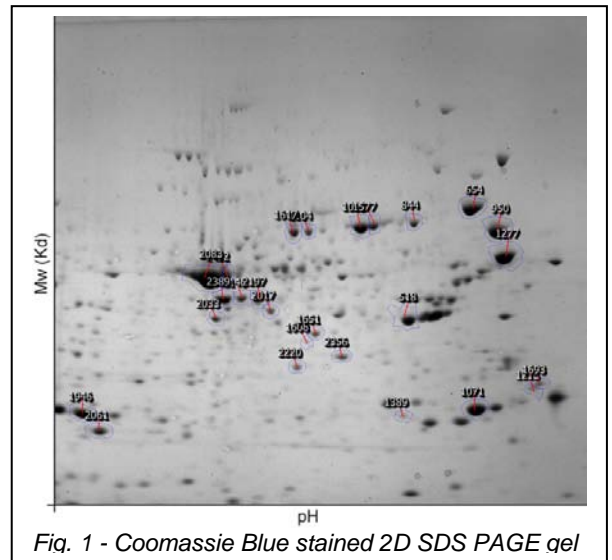
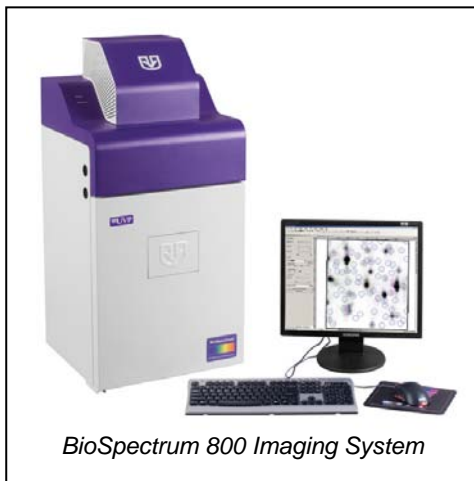


Fig. 1 - Coomassie Blue stained 2D SDS PAGE gel

Applications of 2D SDS PAGE are many (5-14) and include identification of disease or developmental specific proteins. The samples can be analyzed to identify proteins (or constellations of proteins) that are changing in amount (intensity) due to a treatment or disease. Proteins can also be modified after expression in a way that changes their native charge or size, causing shifts in position on a 2D gel. Post translation modifications of proteins (e.g., phosphorylation, glycosylation, etc.) typically lead to changes in mobility, causing a shift in the position that is straightforward to identify through image analysis.

Once visualized by tagging or staining, 2D SDS PAGE separations are imaged with BioSpectrum 800 and the gel image analyzed with Prodigy SameSpots software for number of spots, intensity and position of spots. Prodigy Same Spots greatly simplifies the process of 2D gel analysis (6-14), using a simple guided process that easily produces comparisons between treatment and control samples to look for changes. Due to the inherent variability of the technique, multiple gels must be analyzed for both control and treatment so that any changes can be validated as real and not due to simple gel to gel variability.



BioSpectrum 800 Imaging System

The BioSpectrum 800 system is ideally suited for 2D image analysis:

- 8.3 megapixel camera with resolution better than 50  $\mu$ m
- UV, visible, multiple wavelength excitation and emission fluorescence analysis from 300 to 800 nm
- BioSpectrum 800 with 8.3 mpx camera with 28 mm f/1.8 lens
- High uniformity LED white light plate
- Coomassie Blue high dynamic range (CBHDR) filter VisionWorks®LS software
- Prodigy SameSpots 2D analysis software

## Steps for 2D analysis

Place gel on LED white light plate

With VisionWorks software:

- Set the BioSpectrum 800 camera to 1x1 binning with high quality focus
- Set the filter to Coomassie Blue high dynamic range (CBHDR)
- Adjust the lift height to fill the image frame with the gel
- Adjust exposure time to just saturate the uncovered white light surface
- Adjust the lens at f5.6 to 8 and focus for maximum sharpness
- Save the settings as a template so they can be repeated
- Image all the gels at the same settings
- Save images to archive folder and create duplicate for analysis
- Analyze with Prodigy SameSpots following the simple workflow
  - Image quality control
  - Image alignment
  - Image alignment review
  - Prefiltering and SameSpots analysis
  - View Results and ID interesting spots
  - Report

## Results

Two gels were imaged with VisionWorksLS and analyzed with Prodigy.

**Fig. 1** Coomassie Blue staining 2D SDS PAGE gels. 150 ug of mouse liver was separated by 2D SDS PAGE, imaged with the BioSpectrum 800 system with VisionWorks and analyzed using Prodigy SameSpots software.

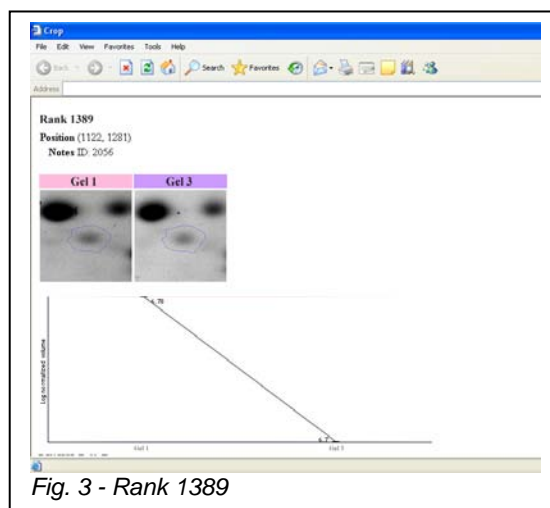
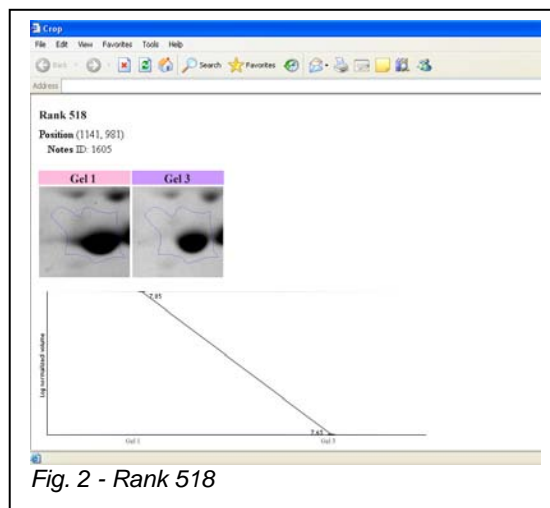
**Fig. 2** Rank 518 Example of a protein that based on Prodigy analysis, increased amount 1.6 fold.

**Fig. 3** Rank 1389 Example of a protein that increased amount 1.2 fold

**Fig. 4** Spot table generated with analysis of the specified proteins in figure 1, showing the rank based on fold change.

## References

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**Fig. 4 - Spot Table**

Rank	Abnva (p)	Fold	Notes	Average Normalized Values	
				Gel 1	Gel 3
518	NaF	1.6	1605	70139497.641	44182270.000
654	NaF	1.5	1159	93570697.267	63074446.000
844	NaF	1.4	1229	20533367.573	14794029.000
950	NaF	1.3	1247	83146913.880	61863940.000
1071	NaF	1.3	2021	78502045.933	60206422.000
1095	NaF	1.3	1244	64342055.366	49646589.000
1104	NaF	1.3	1272	16048729.088	12415801.000
1215	NaF	1.3	1942	6417645.230	5095621.000
1277	NaF	1.2	1331	100154818.651	80848716.000
1389	NaF	1.2	2056	6029312.625	5000785.000
1492	NaF	1.2	1427	36828212.498	31172624.000
1577	NaF	1.2	1239	21893668.961	18943840.000
1608	NaF	1.2	1733	3189354.024	2745145.000
1617	NaF	1.2	1268	24868992.256	21583506.000
1651	NaF	1.1	1694	10791686.600	9427198.000
1693	NaF	1.1	1917	8105527.696	7140607.000

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