Example Analysis Report

Comparison of a 2D Gel and 2D Western blot using SpotMap

Kelly Parkin, Application Scientist

TotalLab Ltd, Third Floor, Dean Court, 22 Dean Street, Newcastle upon Tyne, NE1 1PG, UK
Email: kelly.parkin@totallab.com Tel: 0191 2558899 Web: www.totallab.com

INTRODUCTION

This report demonstrates the use of SpotMap software for analysis of a 2D gel vs a Western blot to compare spot patterns for identification of spots unique to the blot and to calculate relative percentage coverage. The images in this analysis [1] are challenging to analyse due to low colour depth and streaking on the Western blot with low numbers of spots present. These are issues commonly faced in the analysis of 2D gels and Western blots.

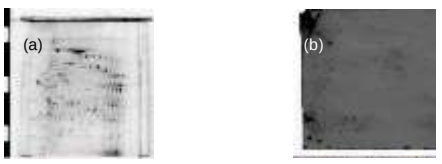
17% coverage was recorded and a total of 711 spots were identified. The analysis was completed within an hour.

SpotMap is the only software developed to be a unique software solution to the challenges associated with analysis of 2D gels and Western blots. It is specifically aimed at analysis of antibody product and process characterisation. It quickly, easily and reproducibly compares different spot patterns and calculates the relative percentage coverage between images. SpotMap is a simple to use and flexible analysis tool for analysis of multiple images and combinations of 2D gels and Western blots.

METHOD

Images

The 2D gel (Gel) and Western blot (Blot) images were uploaded to SpotMap by dragging and dropping into the analysis.



Left: Original Gel image and Right: Original Blot image used in the analysis.

Analysis

In analysis mode, a single spot map is created which identifies the location of all spots in the analysis. Due to the alignment, corresponding spots on different images are in the same coordinate space which allows a single spot map to be created. Each image is overlaid by the spot map and each spot outline is characterised as present or absent from each image.

Automatic spot detection was used on the gel image to create an initial spot map using the following parameters:

- Peak sensitivity = 0
- Smoothing = 0
- Within an area of interest

Following automatic spot detection some manual refinement may be required. Tools available for this include:

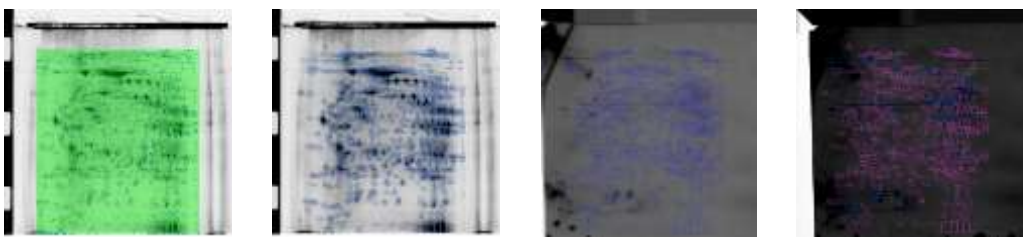
(a) Add spots missed by the automatic detection.
(b) Split two spots automatically detected as one.
(c) Merge fragmented spots.
(d) Freehand draw edits to the spot map.
(e) Delete non-spot features

Any spot added to the spot map, or edited, can be pre-set as present or absent from another image using the New Spot Assignment feature. This prevents an additional spot, present only on one image, needing to be returned to on another image to be set as absent.

Presence or absent categorisation of spots can be set in a number of ways.

(a) Using the Set Present or Set Absent tools to select individual spots or areas of spots to classify.
(b) Setting spot presence whilst editing the spot map using the New Spot Assignment feature. Where any spot added or edited on one image is set as either present only on the active image, hence absent on all other images, or present on all other images.
(c) Using filters to objectively select spots based upon a given criteria to set as present or absent. Spots can be filtered based upon measurements of volume, peak height or area.

In the analysis completed here, spots were edited on the gel image using the new spot assignment of present on all other images. Spots on the blot image were then initially filtered to set any spot with a peak height ≤ 10 as absent from the spot map followed by some manual categorisation with the aid of 3D view.



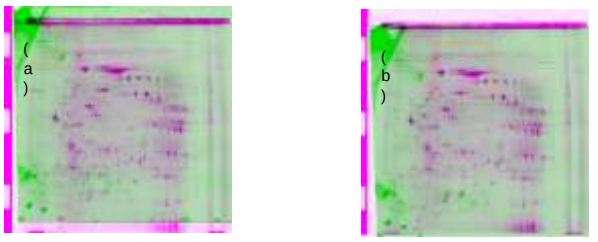
Alignment

Alignment is a unique method that addresses the challenge of comparing two very different spot patterns (commonly seen between gels and blots). It removes the positional variation introduced during the experimental procedure.

Alignment is performed at the pixel level to provide direct and accurate comparison of images. Alignment is completed automatically or manually. Manual vectors are added to assist alignment of images where large positional differences exist.

Alignment can be completed and returned to at any point in the analysis.

In this analysis the Gel was used as the target image to which the Blot was aligned.



Left: The Blot (green) was overlaid on the Gel (purple), (a) before alignment and (b) post alignment.

Right: Key areas for placement of vectors. Manual vectors are identified by red lines and dots.

Results

Percentage coverage, number of spots common between the images and those additional or missing compared to the gel image are presented live as you analyse the images. In the results window and overview of these results, selection the option to view comparison shows the images in a complex colour scheme which colour codes the spots according to common, missing or additional.

Data

All results and data from the analysis can be easily exported using the data access feature. Data is either copy and pasted into its relevant location or dragged and dropped into presentations and reports.

[1] Images supplied by Gathoni Kamuyu, KEMRI-Wellcome Trust Research Programme. *Plasmodium falciparium* immunoproteomic experiments.

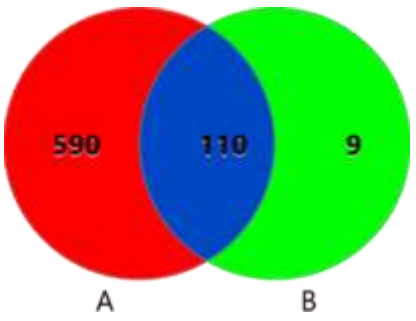
Comparison of a 2D Gel and 2D Western blot using SpotMap

Kelly Parkin, Application Scientist

TotalLab Ltd, Third Floor, Dean Court, 22 Dean Street, Newcastle upon Tyne, NE1 1PG, UK
Email: kelly.parkin@totallab.com Tel: 0191 2558899 Web: www.totallab.com

Results and Discussion

A total of 709 spots were identified across both images, 110 of these were common to both the gel and blot. 590 spots were identified only on the 2D gel and 9 were identified only on the Western blot. Relative percentage coverage of the 2D gel by the Western blot was calculated at 17%.



Venn diagram representing the number of spots unique to each image and common between the images.
A: Gel. B: Blot

Percentage coverage =

$$= \frac{Total\ number\ of\ spots\ present\ on\ the\ blot}{Total\ number\ of\ spots\ in\ the\ spot\ map} \times 100$$

$$= \frac{110 + 9}{590 + 110 + 9} \times 100$$

$$= 17\%$$

Image Quality:

These images flagged QC issues when uploaded to SpotMap.

Both images had 8-bit colour depth, this limited the available pixel values to a scale of 0-255. 16-bit images are recommended for analysis as these increase the pixel intensity scale to 0-65535. See figure 4 for more information.

Areas of the Gel were saturated, this can be seen in Figure 5. Saturation causes peaks to become flattened, reducing the accuracy of the quantitative values and the automatic spot detection. Manual editing is often required to rectify the automatic detection in areas of saturation.

Identification of discrete spots is challenging in areas of these images due to the presence of streaking and non-spot feature, increasing the subjectivity of the analysis. See Figure 6 for an example.

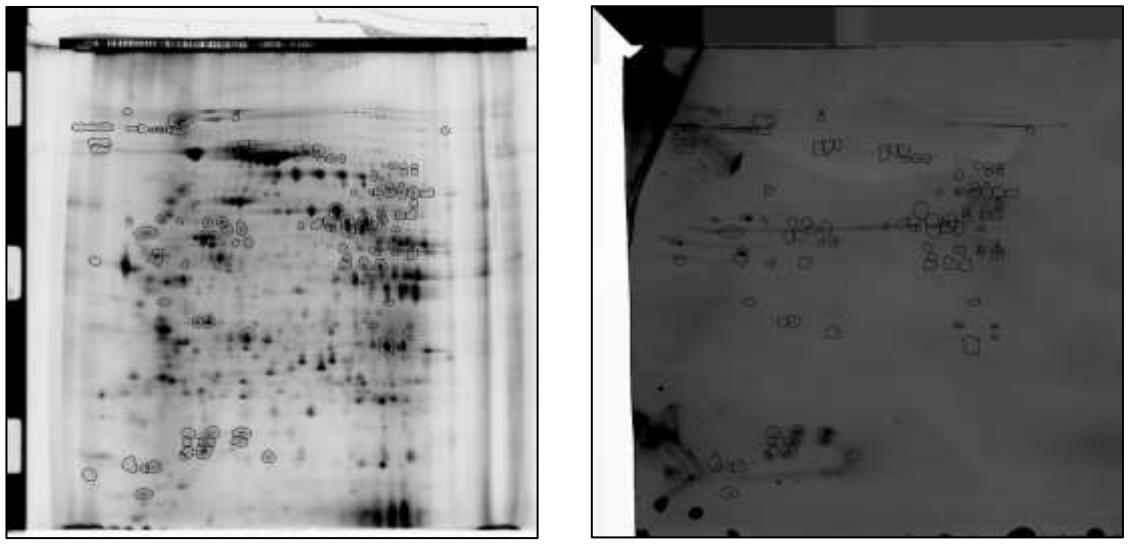


Figure 1. The 110 spots on these images are common to both the 2D gel and Western blot.

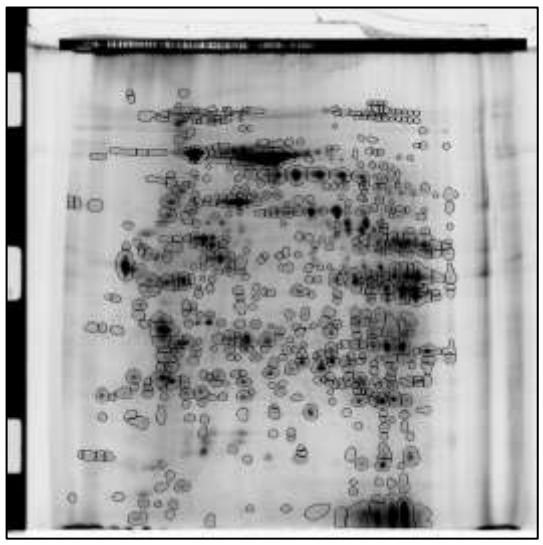


Figure 2. The 590 spots on this image are present only on the 2D gel.

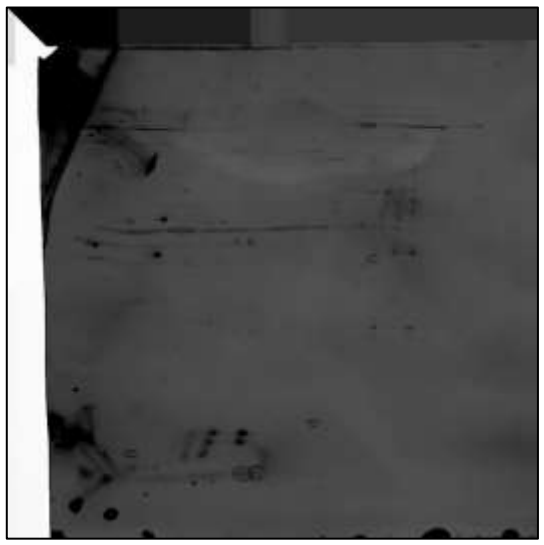


Figure 3. The 9 spots on this image are present only on the Western blot.

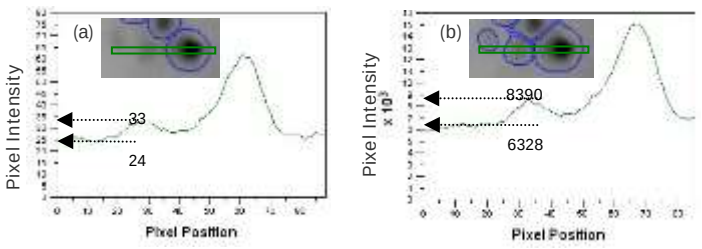


Figure 4. (a) Using 8-bit images spot detection has missed spots due to the low difference in intensity between the spot and background. (b) In the use of 16-bit images the spots have been identified as there is a significant increase in intensity: an increase from 9 to 2062 levels above background intensity.

Figure 5. 3D view of saturated area on the Gel, peaks of spots are flattened therefore the software cannot objectively identify the number of spots present.

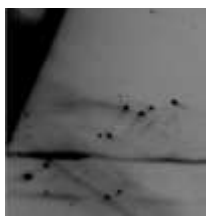


Figure 6. Top left corner of the Blot. Identification of spots is difficult due to the presence of non-spot features, noise and streaking.

CONCLUSION

17% coverage was quickly measured using SpotMap to analyse these images. A total of 709 spots were identified: 590 unique to the Gel image, 9 unique to the Blot and 110 common to both images. Analysis was completed in less than an hour.

The benefits of using SpotMap for analysis of HCP coverage include:

- Multiple 2D gels and Western blots can be analysed in the same simple workflow.
- Automatic quality checks give you confidence in your results.
- Spot detection is automated reducing subjectivity in results.
- Corresponding spots are easily matched through the use of alignment, a unique feature of SpotMap.
- Reproducible and reliable results can be easily obtained through the use of consistent parameters during image analysis.
- The software is easy to use with a quick and flexible workflow.