SpotMap Analysis of Percent Coverage by Direct Comparison of 2D Gel Spot Pattern with 2D Western Blot Features.

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INTRODUCTION

SpotMap has been developed for analysis of HCP coverage for antibody product and process characterisation. It addresses the challenge of comparing two very different spot patterns and calculates percentage coverage of 2D gel vs 2D Western blot.

The images [(1)] used in this report are challenging to analyse due to low colour depth, streaking on the blot and a low number of spots present. These issues are commonly faced in 2D analysis. However the simple workflow of SpotMap allows results to be obtained quickly and objectively.

This report demonstrates the simple workflow of SpotMap that includes image upload and quality check, alignment, spot map creation and identification of presence or absence of spots on the blot, to determine the relative coverage of blot vs gel based on spot numbers. For these images coverage was 24%, (shown to fall within a range of 10% by 3 users).

Contact us to try SpotMap on your own images: kelly.parkin@totallab.com

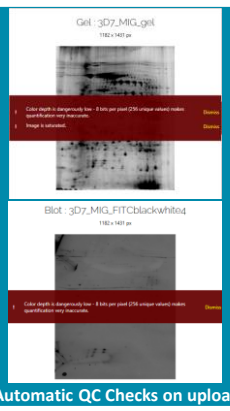
Method

1. Image Upload and Quality Check

2D Gel image (Gel) and 2D Western Blot image (Blot) were uploaded to SpotMap and automatically quality checked.

The key issue was the use of 8 bit images, 16 bit images would increase the accuracy of analysis by increasing the signal of individual spots.

Both images were cropped to remove non-spot features from the analysis.



Automatic QC Checks on upload

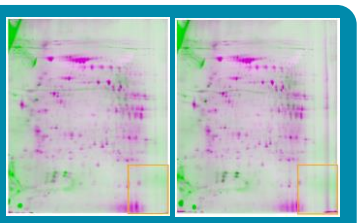
2. Alignment

Alignment is a unique method that addresses the challenge of comparing two very different spot patterns commonly seen between gels and blots.

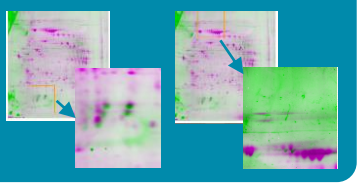
Alignment is performed at the pixel level to provide direct and accurate comparison of images. Alignment is completed automatically or manually. Manual vectors are added to assist alignment of images where large positional differences exist.

4 manual vectors were added to these images.

Overlaid Gel (purple) and Blot (green) images pre (left) and post alignment (right).



Key areas for manual vector addition.



3. Spot Map Creation and Identification of Spot Presence

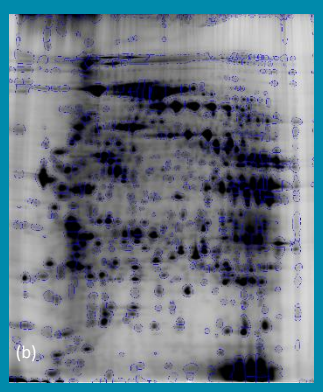
A spot map is created of the fully resolved host cell proteins in the 2D gel, identifying the location of all spots. The spot map is then overlaid on the Blot, where presence or absence of identified spots is recorded to calculate coverage relative to the gel.

3A. Detect Spots

Automatic spot detection creates an initial spot map quickly and objectively.

632 spots were detected in 6 seconds.

The spot detection parameters used were: Peak sensitivity = 0, Smoothing = 2 and removal of background.

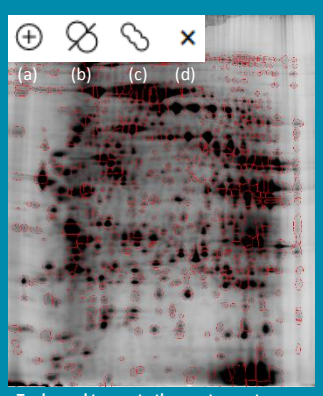


Automatically detected spot pattern of the Gel.

3B. Refine automatic spot map to create the Master Spot Map

The automatically detected spot map is manually edited to ensure accuracy using the available tools:
(a) **Add** spots missed by the auto detection
(b) **Split** 2 spots automatically detected as 1.
(c) **Merge** fragmented spots
(d) **Delete** non-spot features

The final master spot map represents the position of all spots present on the gel.



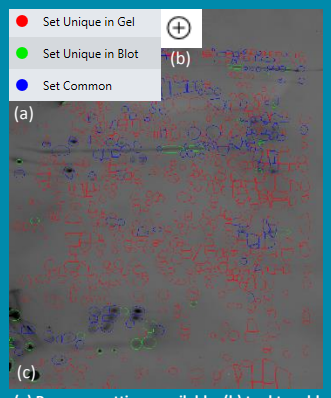
Tools used to create the master spot map. Main image: final master spot map.

3C. Determine Coverage

Alignment allows the master spot map to be applied directly to the blot. Spots common to both Gel and Blot will appear in the same coordinate space.

Spots are recorded as:
- Unique to Gel
- Unique to Blot
- Common

Additional spots present only on the Blot may be added using the specialised add tool.



(a) Presence settings available. (b) tool to add additional spots to the map. (c) complete spot map with presence set.

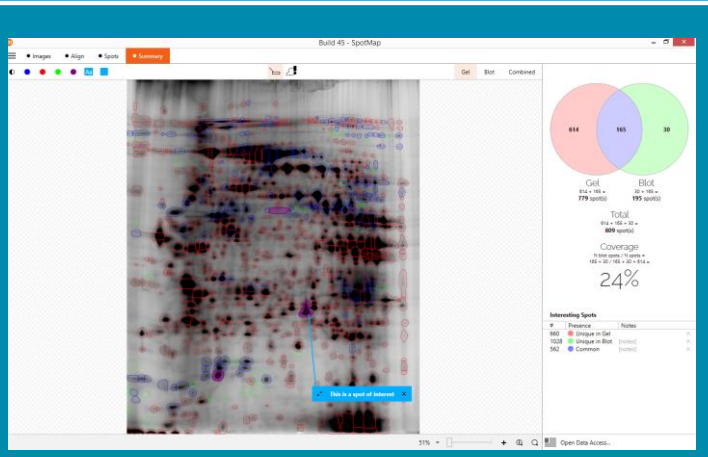
4. Summary

The summary page displays the results of the analysis and allows annotation of the spot map with easy export of data directly into reports and presentations.

- Coverage is calculated as a percentage of total spots represented on the Blot. Unique and common spots are easily identified by colours.

Coverage % = N Blot Spots / N Total Spots x 100
Blot spots = Unique to Blot + Common
Total = Unique to Blot + Unique to Get + Common

- On the spot map, annotations can be made and spots can be identified as “interesting”. Interesting spots are listed and notations can be made beside these.
- The Data access window allows for simple drag and drop placement of data from the experiment directly into reports and presentations.



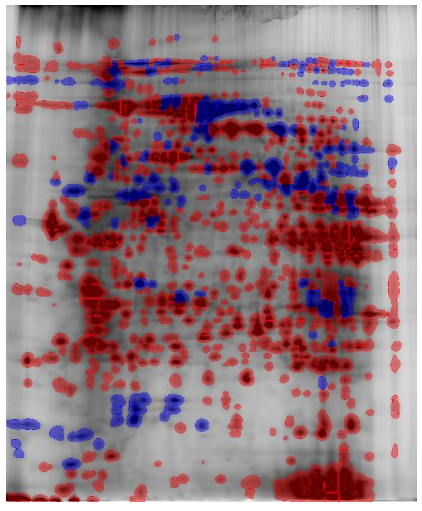
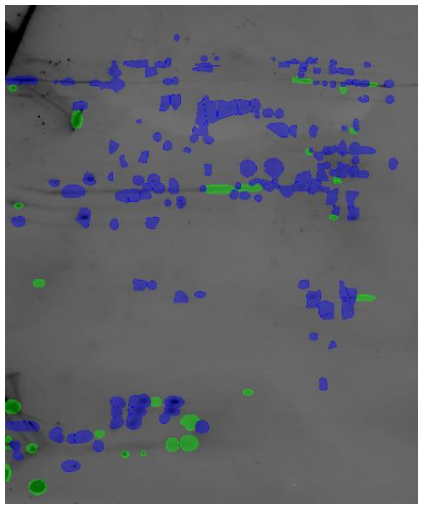
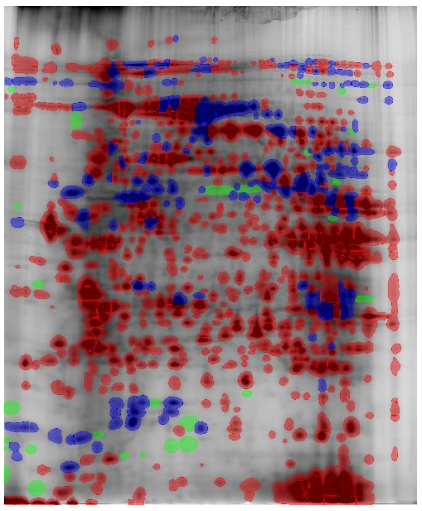
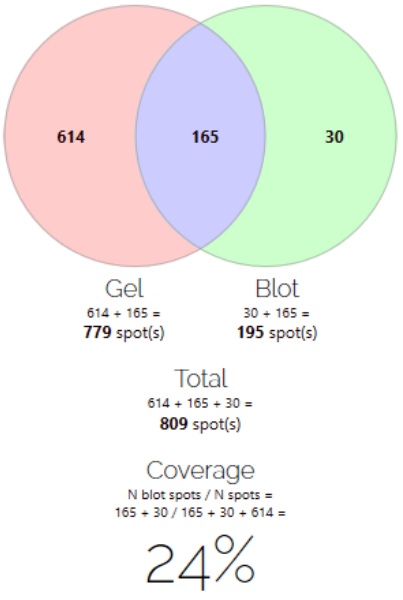
The analysis is summarised in the summary screen. Data can be exported directly from the software into reports and presentations using the data access window.

(1) Gathoni Kamuyu, PhD Student, KEMRI-Wellcome Trust Research Programme, *Plasmodium falciparum* immunoproteomic experiments.

For more information on the workflow of SpotMap get in touch: info@totallab.com

■ Spots Unique to Gel ■ Spots Unique to Blot ■ Spots common to Gel and Blot.

Percent coverage based on spot number = total number of spots on Blot/ total number of spots *100

Gel Image	Blot Image	Combined Image on Gel	Summary of results presented by SpotMap
			
Gel only spots: 614 **Common spots: 165** **Total spots on Gel: 779**	**Blot only spots: 30** **Common spots: 165** **Total spots on Blot: 195**	**Spot pattern of Gel only, Blot only and Common spots.** **Total spots in map: 809**	

The images used are challenging, however they are examples of many of the challenges faced by new users of 2D analysis with a non-optimised process.

- These were 8 bit images, limiting the pixel values to a scale of 0-255, some spots may fall below detectable levels of the software and will require manual addition to the spot map. Use of 16 bit images would increase the pixel intensity to a scale of 0-65535 (figure 1).
- Areas of the gel image were saturated, this can be seen in the 3D image below where the peaks of some spots have been flattened. This prevents to automatic detection from identifying individual spots, reducing the accuracy of the automatic spot detection. Manual editing can split spots detected as one (figure 2).
- Alignment is challenging for all gel vs blot analysis as there are often few common features between the images. This challenge is overcome by use of manual vectors.
- Identification of discrete spots is challenging in these images as there are areas of streaking on the blot and presence of non-spot features on both images (figure 3), each images has different background intensities, affecting the visibility of spots. An optimised gel running, blotting and imaging process would minimise these issues.

Using the simple workflow of SpotMap analysis of these images took less than 1 hour.

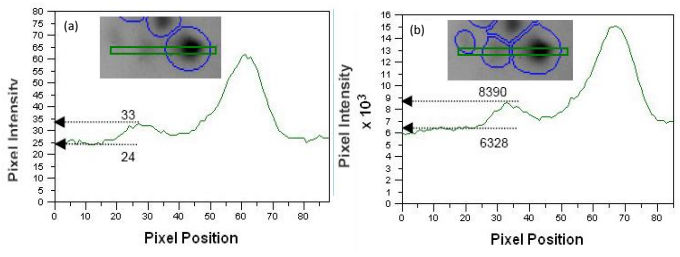


Figure 1. (a) Spot detection has missed spots due to the low difference in intensity between the spot and background noise. In the use of 16 bit images (b) the spots have been identified as there is a significant difference in intensity. An increase from 9 to 2062 levels above background intensity.

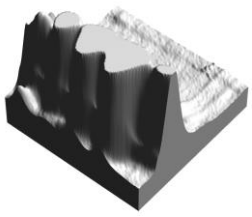


Figure 2. 3D view of saturated area on gel image, peaks of spots are flattened therefore the software cannot objectively distinguish presence of one spot or more.

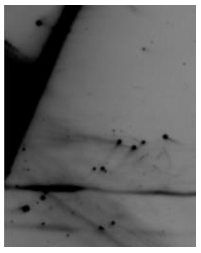


Figure 3. Top left corner of blot image, identification of spots is difficult to distinguish due to the presence of non-spot features, noise and streaking.

CONCLUSION

SpotMap has been specifically developed to measure the relative coverage of 2D gel vs 2D western blot images, even with the use of challenging images, as seen above.

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

- **Automatic quality checks and visualisation of image issues**, gives 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 simple and quick work flow.**

24% coverage was calculated by SpotMap using these challenging images. A total spot map of 809 spots was identified, 614 are unique to the Gel and 30 are unique to the Blot.

Try SpotMap on your own images. Contact us today: kelly.parkin@totallab.com