



What problems does SpotMap solve?

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Introduction

What problems does SpotMap solve?



SpotMap addresses the challenges of analysing 2D gels and Western blots. Images are quality checked on upload to address any potential challenges with the analysis. Positional variation in the spot patterns of the 2D gel and blot can make identification of corresponding spots challenging, alignment helps to overcome this. A single spot pattern is created to easily identify the corresponding spots on each image and results are clearly presented for easy reporting.

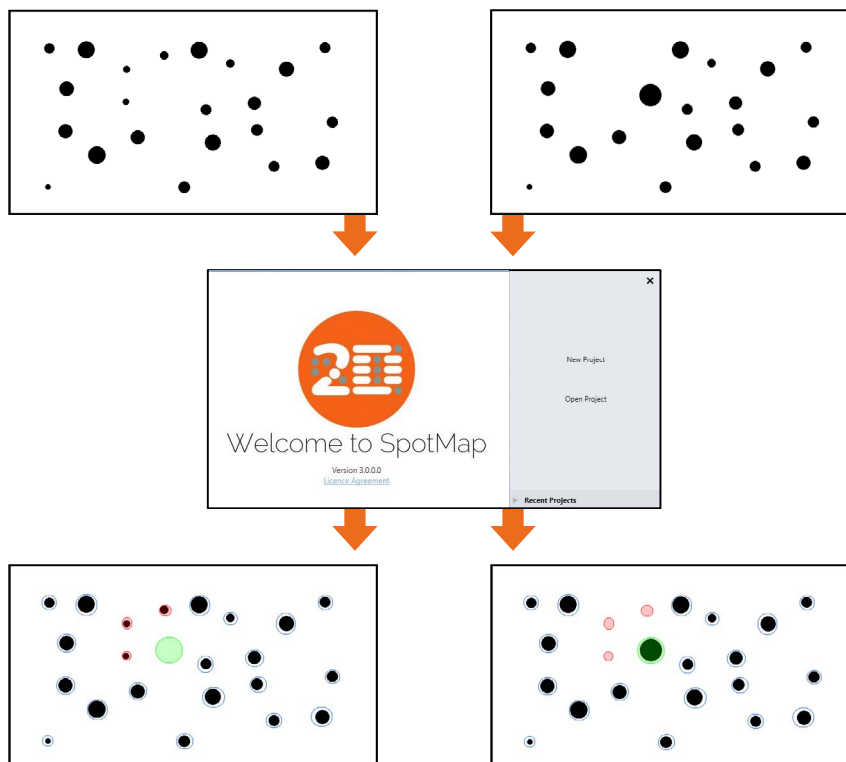
SpotMap can:

- Identify spots unique to an image in an analysis.
- Measure relative coverage of a 2D gel by a Western blot.
- Compare 2D gels and Western blot to identify those proteins which the antibody recognised.
 - Be used to characterise a host organisms proteome or an anti-HCP antibody.
 - Compare different protein profiles of strains.
 - Monitor proteins through a purification process.
 - And more...

How does SpotMap solve these problems?



SpotMap has a simple, flexible and reproducible workflow which overlays corresponding spots in the analysis and creates a single spot map. Spots in the map are then categorised as present or absent from each image and used to calculate percentage coverage and identify common or unique spots.



87% coverage of image A by image B.

19 Spots Common.

3 Spots Missing (unique to image A).

1 Spot Additional (unique to image B).

(When image A is the base image).

What problems do the features of SpotMap overcome?

Can I check my image quality?

✓ Yes!



When adding images to the project they are checked for quality and any issues/notes are highlighted for you.

Can I edit my images?

✓ Yes!



If your images require minor changes (flipping etc.) then you can edit them within the software without changing the original images.

Can I account for gel/blot distortions?

✓ Yes!



Alignment makes it possible to accurately compare images by removing the positional variation introduced during the gel running, blotting and imaging process.

Can I identify spots of interest?

✓ Yes!



Spots are categorised as present or absent during the analysis, this allows spots Common, Additional and Missing from an image comparison to be easily identified.

What problems do the features of SpotMap overcome?

Can I measure percentage coverage of 2D gels and Western blots?

✓ Yes!



Based upon the categorisation of spots, percentage coverage of one image vs another is calculated.

Can I compare spot measurements?

✓ Yes!



All spot measurements are available for export or may be viewed in the table within the software. Spot measurements include volume, peak height, area, background and position. If pI/MW information has been added to the image the estimated values are also available.

Can I calculate spot coverage?

✓ Yes!



A spot map is created of the fully resolved host cell protein. The presence of spots expressed on the blot is then used to calculate the relative coverage of the Western blot vs the 2D gel.

Can I export results?

✓ Yes!



All the data from the project can be easily exported from the program using the Data Access window or using the clipboard functions. .csv files of the numerical data are easily exported for all spots on all images, or for selected spots only. Images can be exported by copy and paste or exported as a .png file.

Images

What is the Images mode?



Images mode allows you to upload the images to the project. On upload the images are quality checked and minor image edits can be performed.

What is Image QC?

When adding images to the project they are quality checked and any issues/notes are highlighted for you immediately before any further analysis is attempted. Image QC flags any potential issues with:

- Bit-depth
- Image file size
- Saturation
- Manual editing of the images

Poor image quality can make analysis of your gels and blots difficult and may reduce the objectivity of your analysis.

For more information on Image Quality in TotalLab software visit: www.totallab.com/imagequality and download our Image Capture Guide.

What are the benefits of using high quality images?

Quantification of spot intensities may seem less important when only looking at spot numbers and coverage results. However, good quality images also improve:

- Spot Detection
- Filtering options when selecting small/low spots to remove
- Definition of spots on the blot (no saturation)
- Automatic alignment algorithm

Images

Can I edit my images in the software?

✓ Yes!



Quick edits are available below each image to invert the colour intensities and flip or crop the images. More edits can be performed in the image editor window, accessed by right clicking on the image and selecting "Edit" from the menu. It is recommended that image edits are performed within the software as this will not affect the raw data of the images.

What problems does image editing solve?



Images which are different in size, shape and orientation are hard to compare directly. Poor image quality can make analysis of your 2D gels and Western blots difficult and reduce the objectivity of your results.

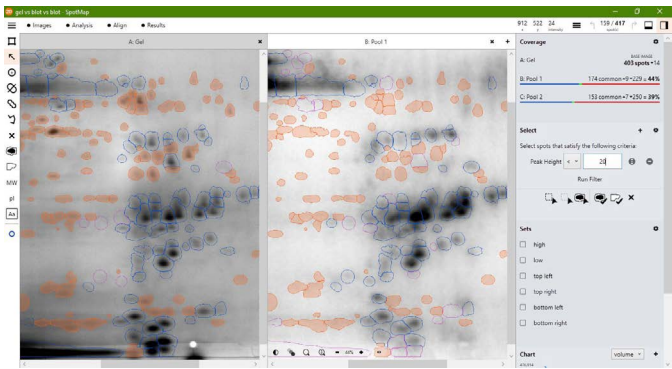
What edit options are available?



- Flip images horizontally and vertically
- Rotate images 90 degrees clockwise and anti-clockwise
- Invert image
- Translate image
- Crop image
- Rotate image a variable angle
- Resize images to same size

Analysis

What does the analysis mode do?



?

In the analysis mode, a master spot map identifying the location of all spots in the project is created and spots on each image are categorized as present or absent from this map. The present or absent settings are used to identify spots common, missing or additional to the images in a comparison and measure % coverage.

Why is a single spot map used for the analysis?

?

Alignment places corresponding spots in the same coordinate space on the images, this allows a single spot map to be used. Using a single spot map allows you to directly compare matching spots/features between images and easily identify spots absent from that given image.

How does identifying spots as present or absent allow results to be calculated?

?

The present or absent categorizations from the spot map allow comparisons of images to be made. When any two images (the base and the secondary) are compared the spot presence/absence settings are used to determine which spots are Common, Missing or Additional and are used to calculate percentage coverage.

	Base Image	Secondary Image
Common	Present	Present
Missing	Present	Absent
Additional	Absent	Present
Absent (Not included in calculation of spot coverage. Spots classified as absent should be present on another image in the analysis).	Absent	Absent

What is spot coverage and how is this calculated?

?

Spot coverage is the percentage of spots in the comparison being made which are present on the secondary image. Percentage coverage is calculated against the chosen base image using the calculation:

Percentage coverage

(Common + Additional)

x 100

(Common + Missing + Additional)

Analysis

What is a base image?



The base image is the image against which the others are compared. The base image can be selected to be any other image in the analysis. The term secondary image refer to any image compared to the base image.

What does automatic spot detection do?



Using advanced imaging techniques an initial spot map is created of the selected image within the set parameters of smoothing and peak sensitivity. A rectangular or elliptical region of interest can be added to the analysis to target the spot detection.

Why are spots detected on only one image?



Only one spot pattern needs to be detected as corresponding spots are in the same coordinate space on all images following alignment. The chosen image should be the one with the most spots present or the most representative spot pattern.

Why are some spot edits required?



Sometimes the automatic spot detection results need to be refined. Spot outlines around the edge can be removed. Spot outlines in the middle can be added, deleted, merged or split. Additional spots can be added to the map. Our tests have shown that you can have different levels of editing (little or a lot) and still get similar coverage results. It is key to be consistent so that you get similar results across experiments. Using the new spot assignment feature when editing spots will aid in categorising spots by pre setting the presence or absence of a spot on all other images.

Why select spots using clauses?



The filter option is usually used to objectively select spots you may wish to remove from the spot map or categorise as present or absent from the image. A filter clause can be created based upon a measurement of area, volume or peak height and set based on a specific value, a selected spot or chosen from a sliding scale. Selected spots can also be used to create a spot set.

What is a spot set?



A spot set can be created from a spot selection, manually chosen spots or predefined areas, i.e. quadrants. Results and spot measurements can be collated for each spot set.

Can I add molecular weight and pH markers?



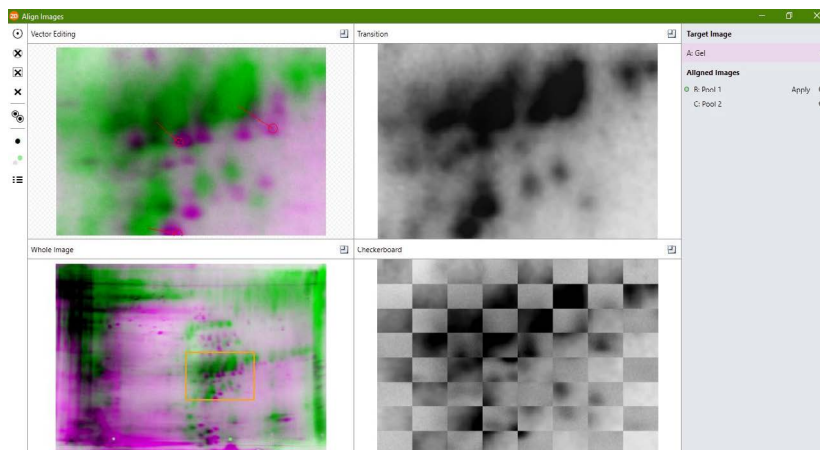
Yes!



Markers can be added to the map to identify the MW markers.
A linear or non-linear pl strip can be added to the map.

Alignment

What is Alignment?



Alignment takes two images and overlays them in the same coordinate space so that a spot on the image to be aligned will be in the same location as the matching spot on the target image.

What problem does it solve?

Alignment makes it possible to accurately compare images by removing any positional variation introduced during the gel running, blotting and imaging processes.

Why is alignment better than spot matching techniques?

Using separate spot detection on each image and then matching each spot between the images is very inefficient. Alignment makes it possible to overlay spots from one image to another, placing them in the correct location.

How is alignment different to warping?

Warping and alignment both aim to remove the positional variation introduced during the gel running, blotting and imaging process. However warping is only accurate to the spot level whereas alignment is accurate to near pixel level.

Do I need to align DiGE images?

Alignment is not necessary for DiGE images. However, for low MW proteins there may be a shift due to the different dye, in this case alignment can be used.

Alignment

Can I "fix" alignment after spot detection?

✓ Yes

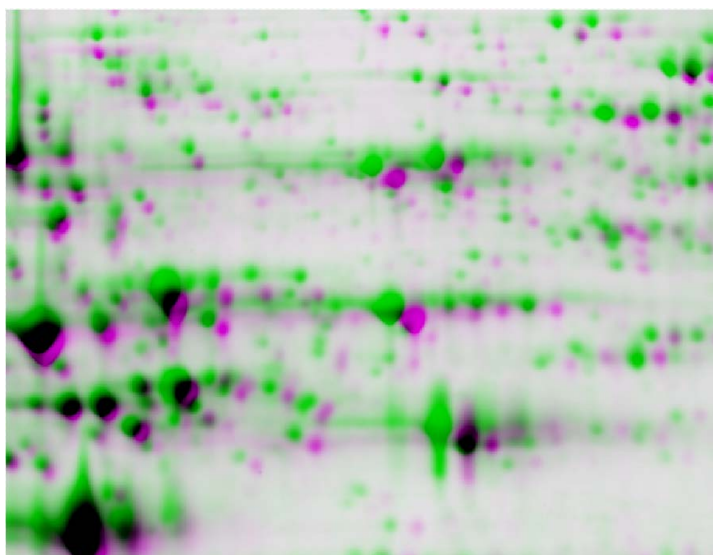
?

The alignment window can be opened at any point in the project to align or correct the alignment of an image. Once the "Apply" button has been clicked the alignment will update. The images can be initially aligned prior to or following spot detection.

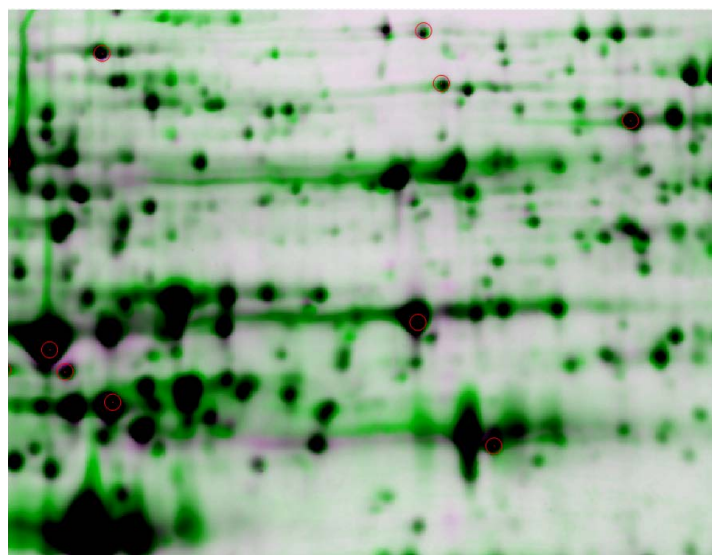
How does Alignment work?

?

Automatically generated or manually added vectors between features on each image are used to create a map between the images. If the images have similar spot patterns then this can be fully automatic using our fast alignment algorithm.



Overlay Before Alignment



Overlay After Alignment

What is the difference between automatic alignment vectors and manual vectors?

?

Automatic alignment attempts to look at the spot patterns between the images and match up similar spots/features and puts in automatically generated vectors. Manual vectors are added by users to features that they think are matched. These can be added before automatic alignment, which helps the algorithm, after automatic alignment to fix areas or simply on their own if automatic alignment fails or is difficult.

What does the results screen show?



The results screen presents an overview of the percentage coverage and numbers of spots common and additional to the base image. There is a small heat map which identifies where the spots common, additional and missing are in relation to the whole image, selecting "View Comparison" will open the base image and selected image in the analysis window in the colour scheme of Common, Additional and Missing spots. Results for spot sets can also be viewed in the results screen.

Can I export data?



Yes!



Using the Data Access feature, found from the main menu, all data from the analysis can be easily exported by dragging and dropping into reports and presentations or files.

What data can I export?



- Results of percent coverage
- Number of spots common, additional and missing between images.
- Colour coded spot maps overlaid on the images identifying the location of Common, Missing and Additional spots.
- CSV data file containing all measurements of all spots for all images or a spot set.
- All analysis parameters used in the analysis.
- Manually added images.

Images throughout the analysis can be added to the data access by right clicking and selecting "Add to Data Access".

Can I annotate the image?



Yes!



You can add text labels to the spot map in the analysis screen, these can point towards certain features of the map which are of interest.

Disclaimer

All material in this brochure has been written by collating information from various sources. Where possible these sources have been cited. It is a guide and not a protocol or standard operating procedure. It may not give optimal results for individual samples and systems. You should check parameters specific to your own sample, instruments and image capture software. Common best practice is to run pilot experiments to optimise sample handling, gel running, image capture and image analysis.

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