



Welcome to SpotMap

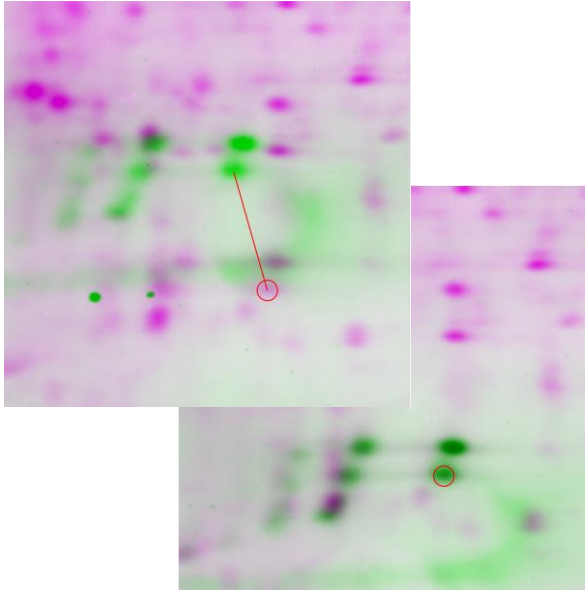
Version 5.0.055

For more information, please email michael.hutchins@totallab.com

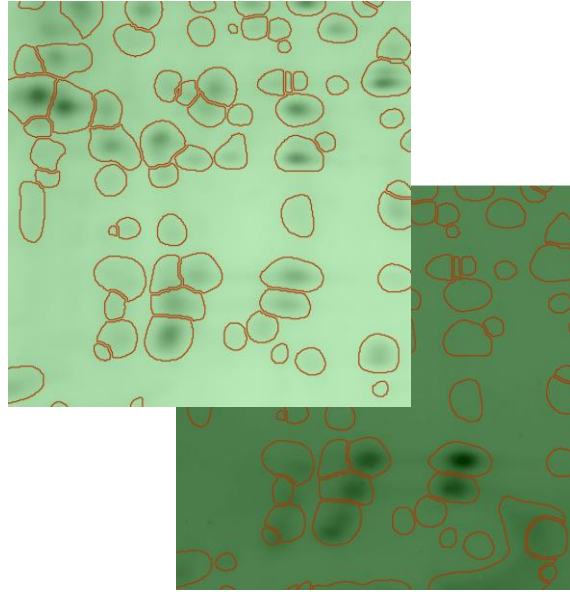
How does SpotMap work?

For more information, please email michael.hutchins@totallab.com

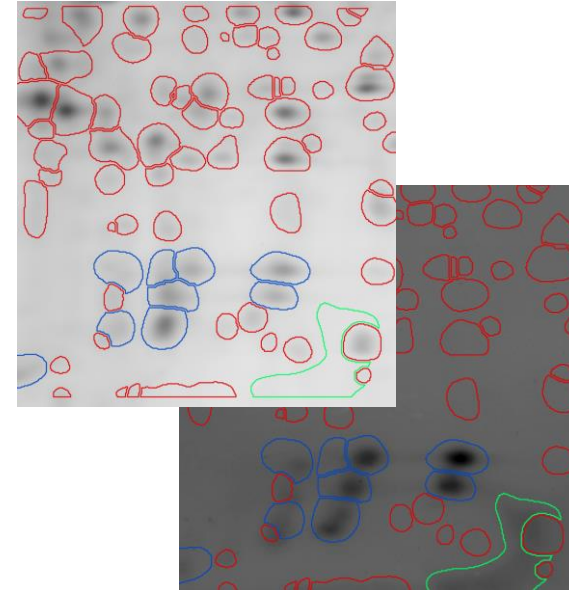
Simple 3 stage process



(1) Import and align images



(2) Detect spots



(3) Assign presence/ absence

Useful Information:

SpotMap v5 is useful for:

- HCP coverage analysis comparing a gel and Western blot
- Protein purification studies comparing multiple gels
- Process improvement studies comparing gels from old and new processes
- Studies comparing Western blots using multiple anti-sera and exposure times
- Identification of product reactions with anti-HCP antibody

Full 21CFR part 11 support is available through the optional Version Control module:

- Electronic sign-in of multiple users with different permissions
- Full traceability at all stages of analysis
- Electronic sign-off and approval
- Data integrity ensures data has not been modified throughout analysis
- Full audit trail and access log

For more information, please email michael.hutchins@totallab.com

Useful Information:

What do I need to run SpotMap v5?

- Windows 7+ (64-bit)
- Intel i5+ processor (or equivalent)
- 8Gb+ Ram
- Recommended 1080p Screen

Recommended Image Capture:

- 16-bit greyscale TIF images
- 100 micron or 300dpi
- Dimensions 2500 x 2000 pixels

For more information, please email michael.hutchins@totallab.com

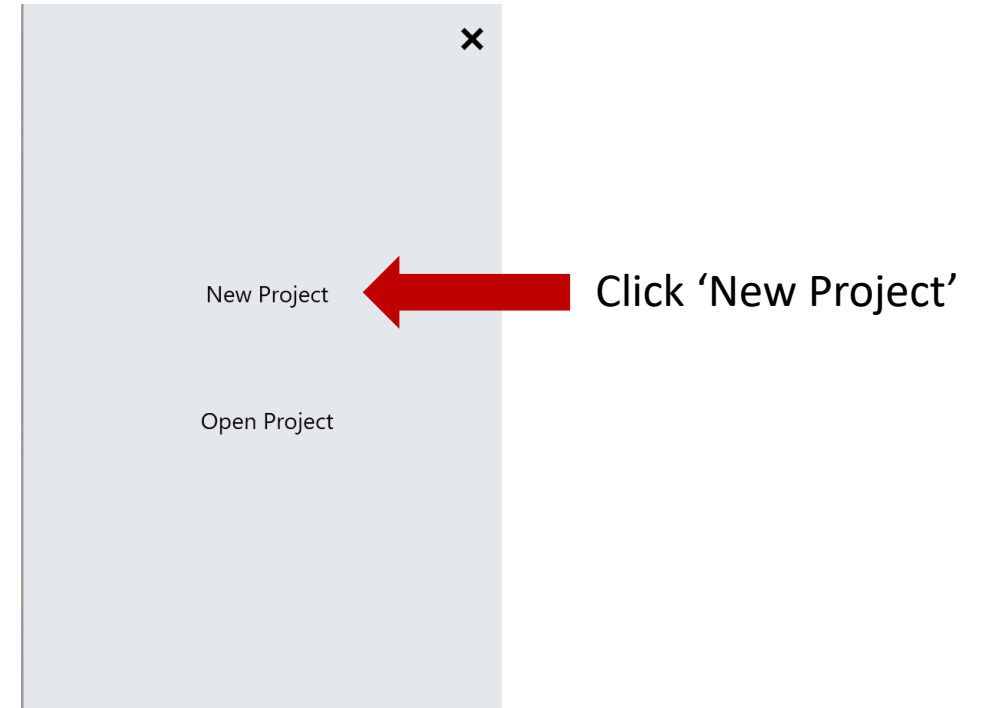
Walkthrough

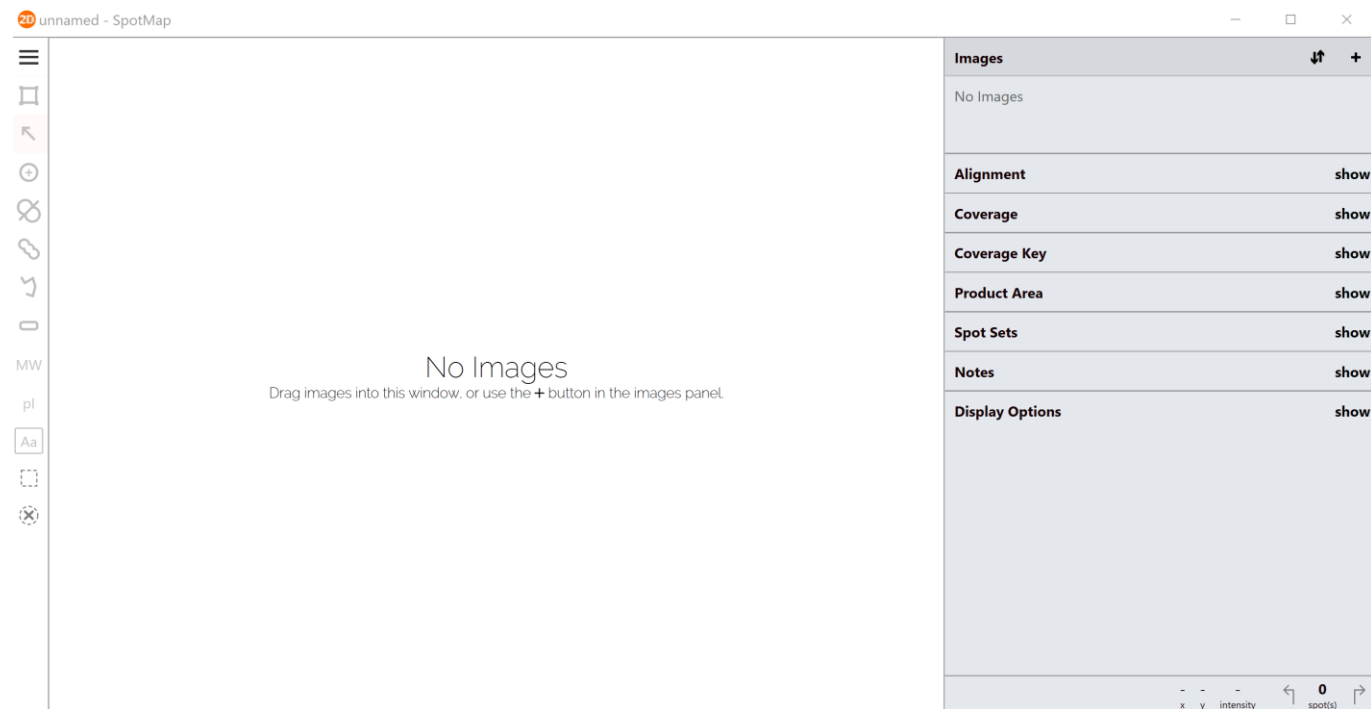
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Welcome to SpotMap

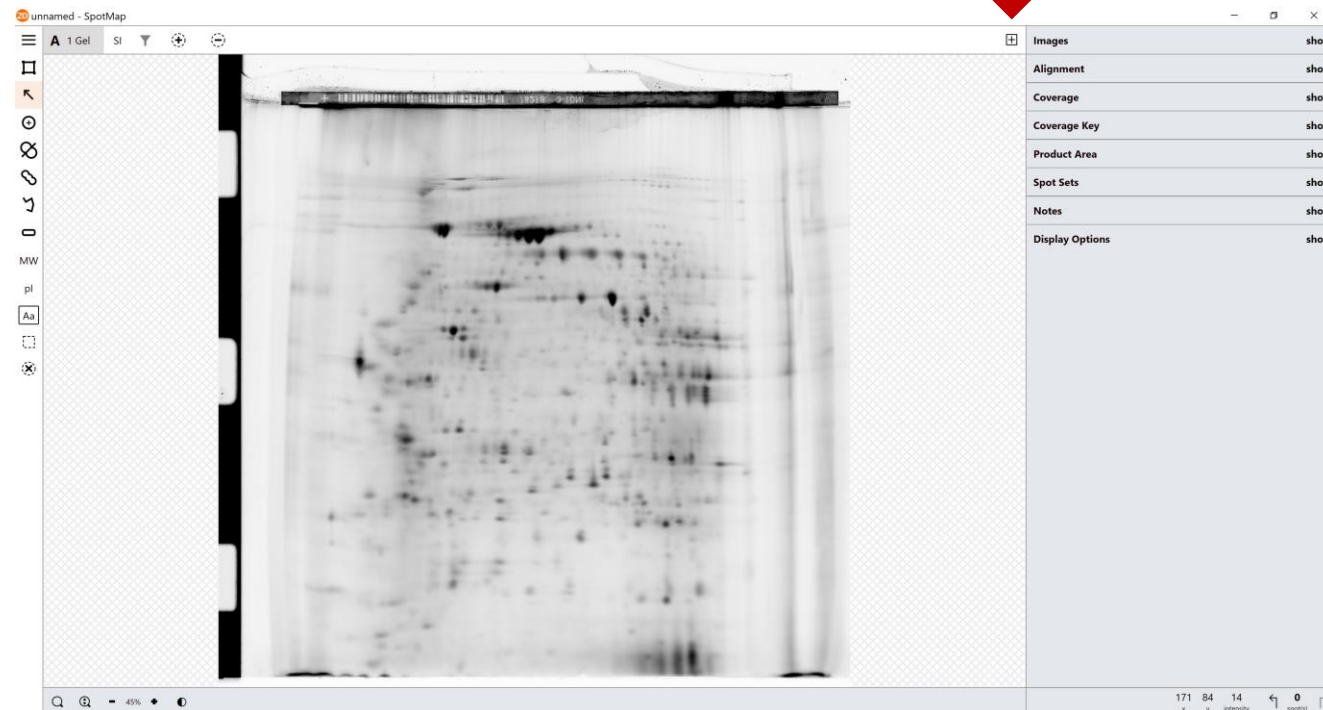
Version 5.0.055





Click to add images

Click to view second image



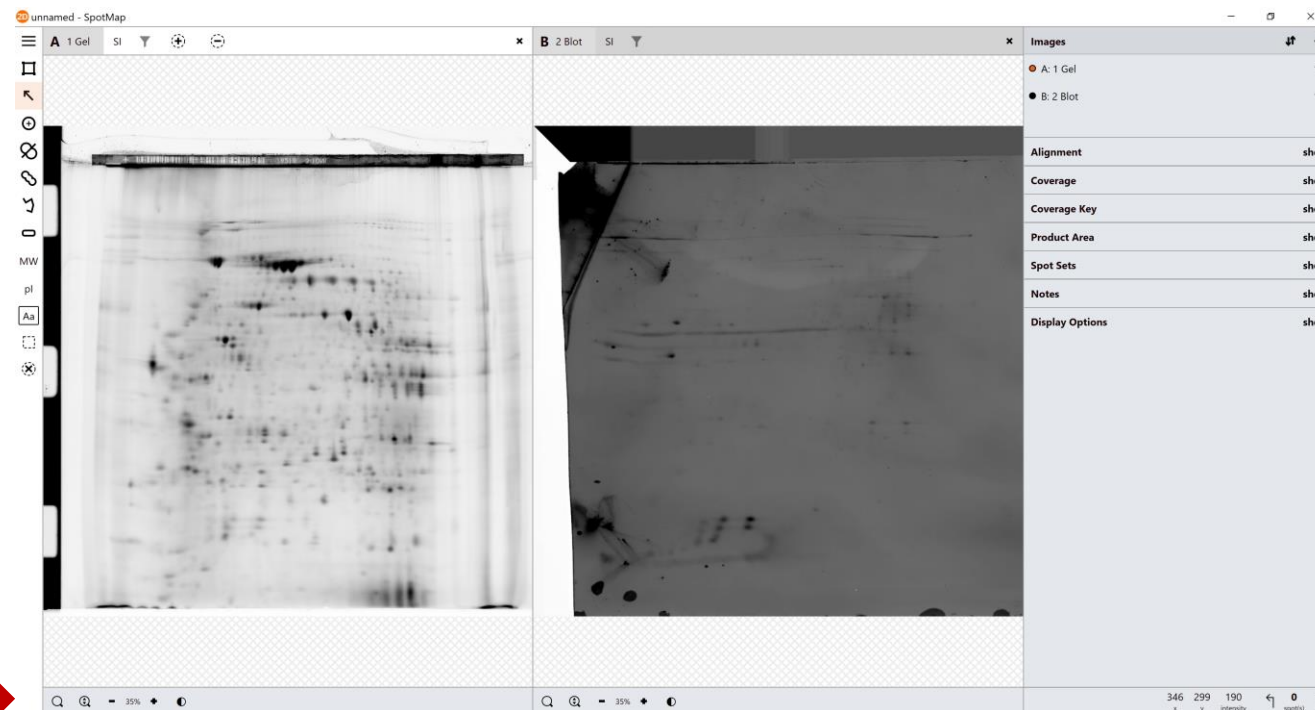
For more information, please email michael.hutchins@totallab.com

Viewing your data

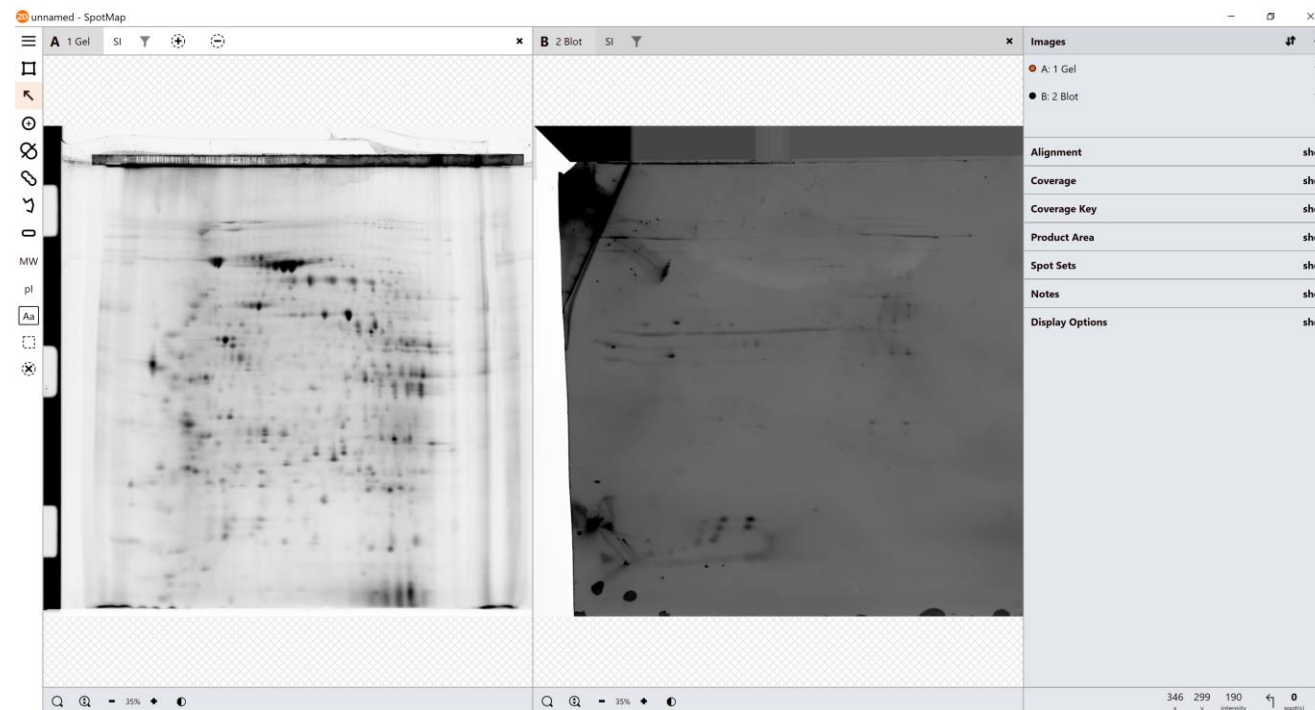
For more information, please email michael.hutchins@totallab.com

Zoom controls:

- Manual Zoom
- Maximise Image
- Zoom in and out

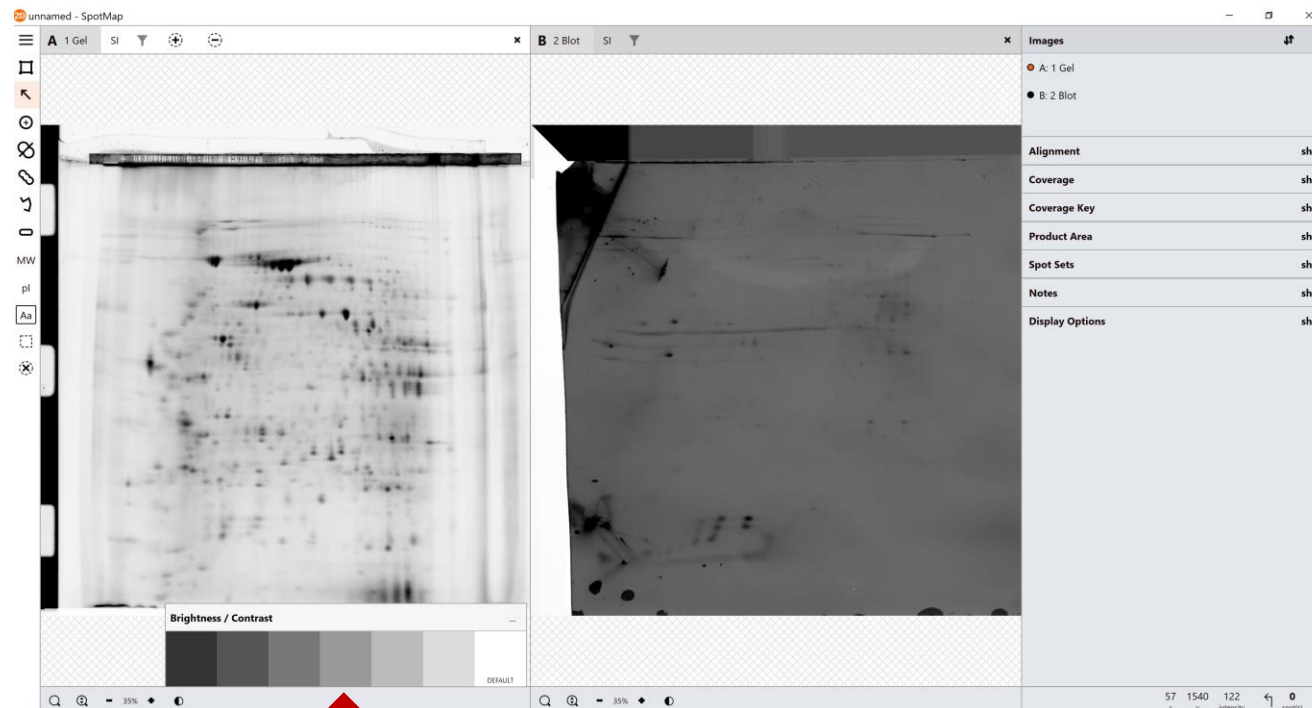


For more information, please email michael.hutchins@totallab.com



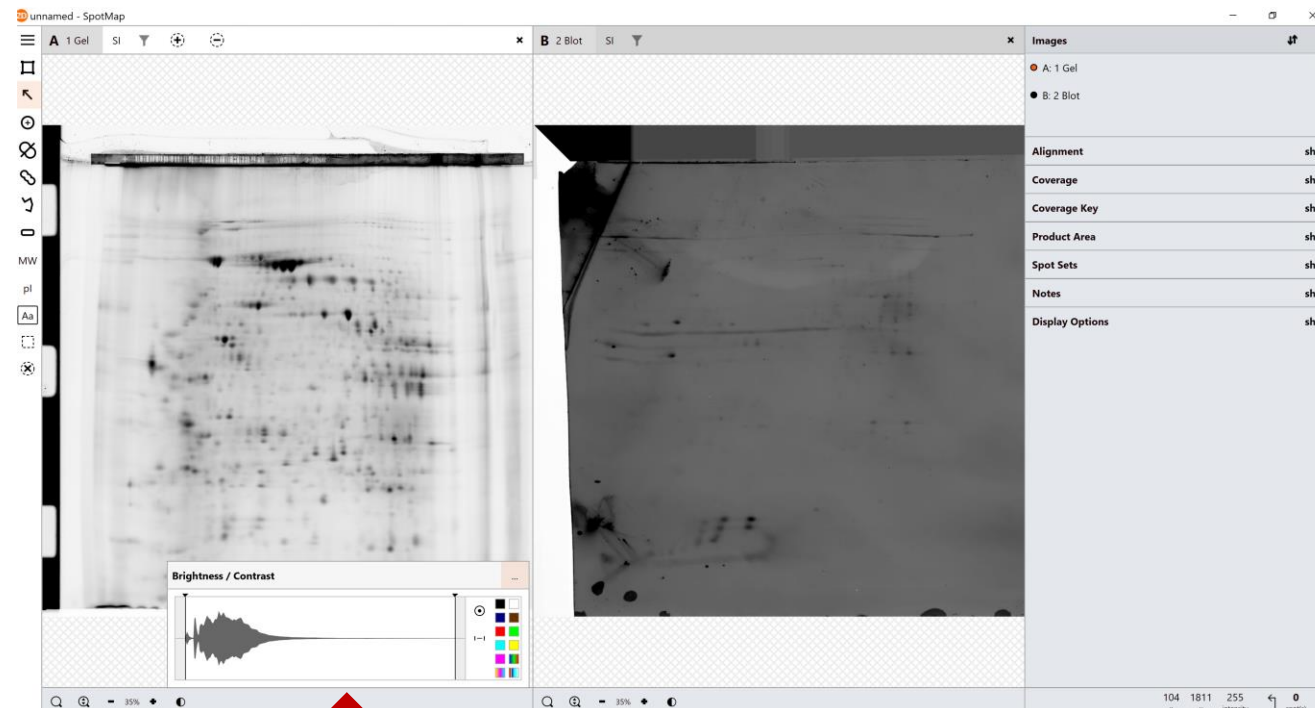
Adjust image contrast
and add false colour

For more information, please email michael.hutchins@totallab.com



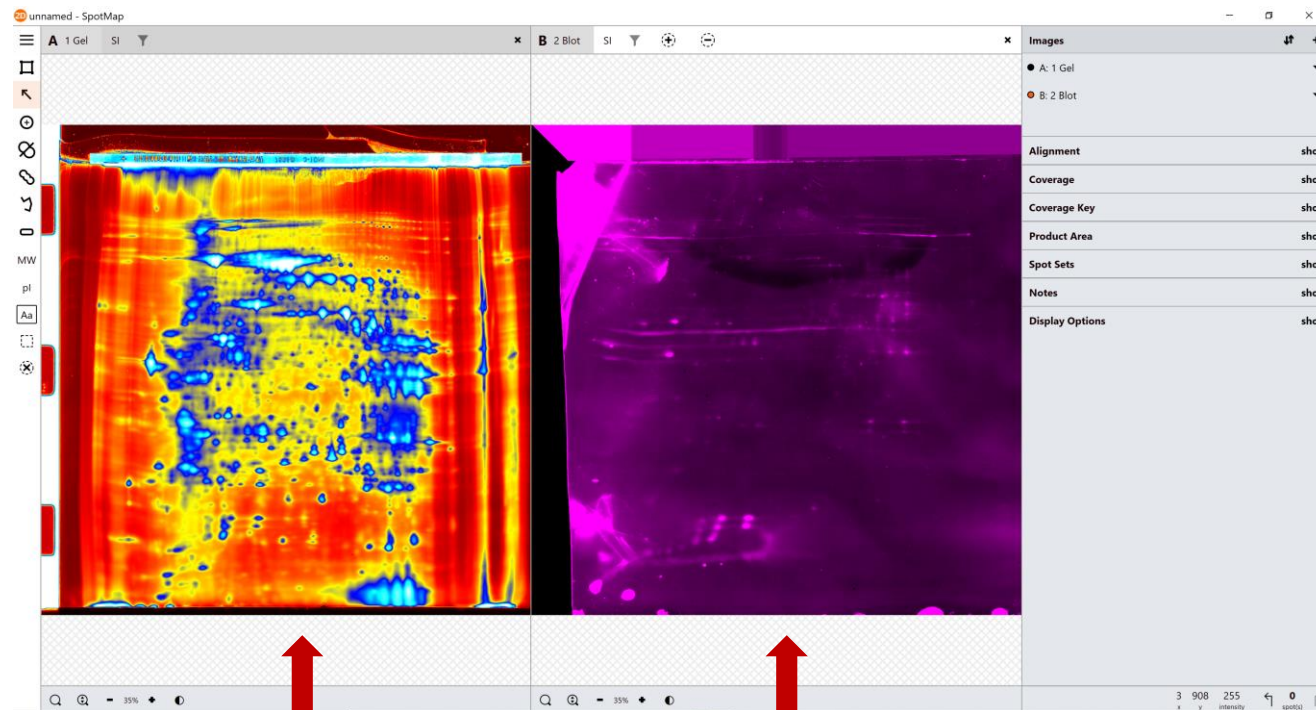
Adjust image contrast

For more information, please email michael.hutchins@totallab.com



Advanced image contrast
adjustment and false
colour render

For more information, please email michael.hutchins@totallab.com

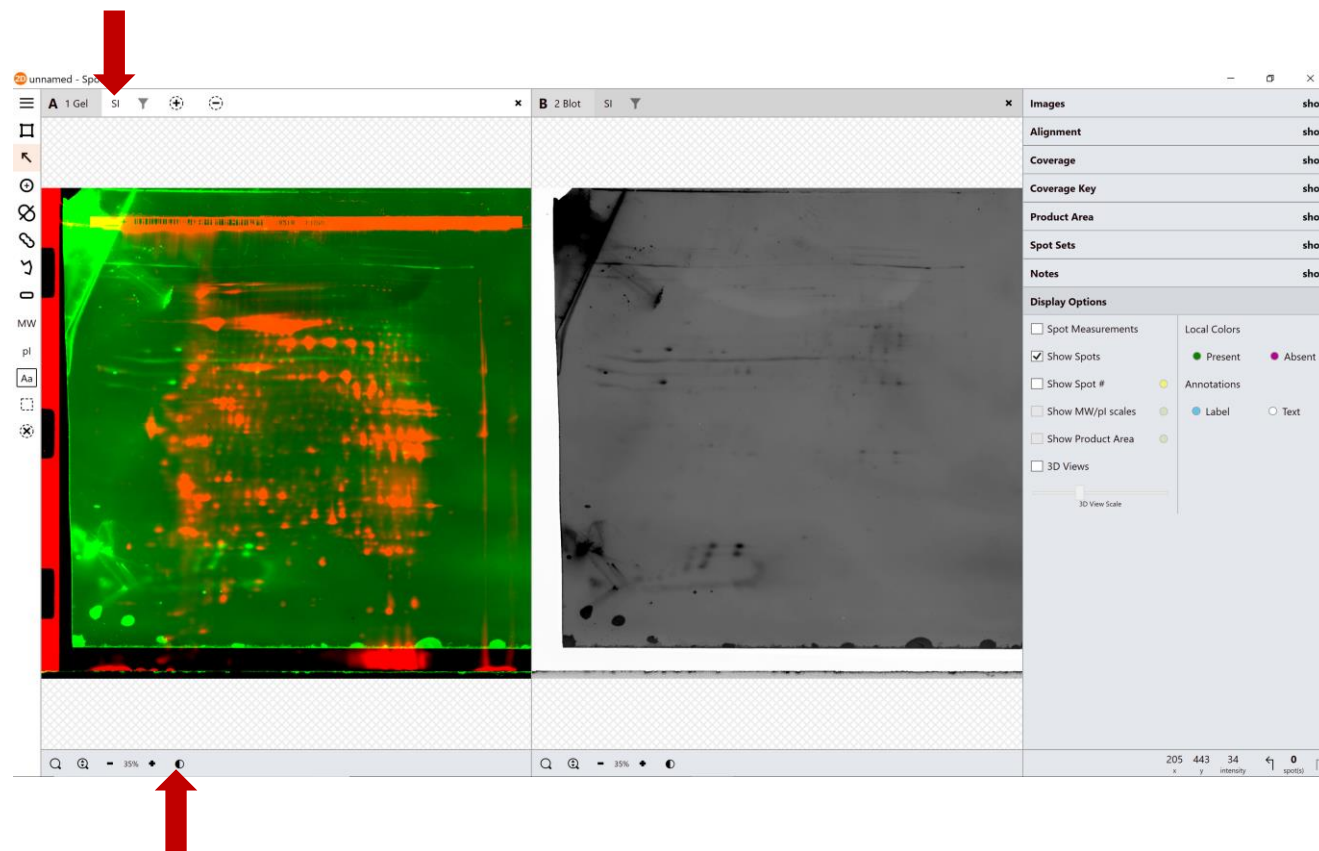


Heatmap 3 applied
to gel image

False colour applied
to blot image

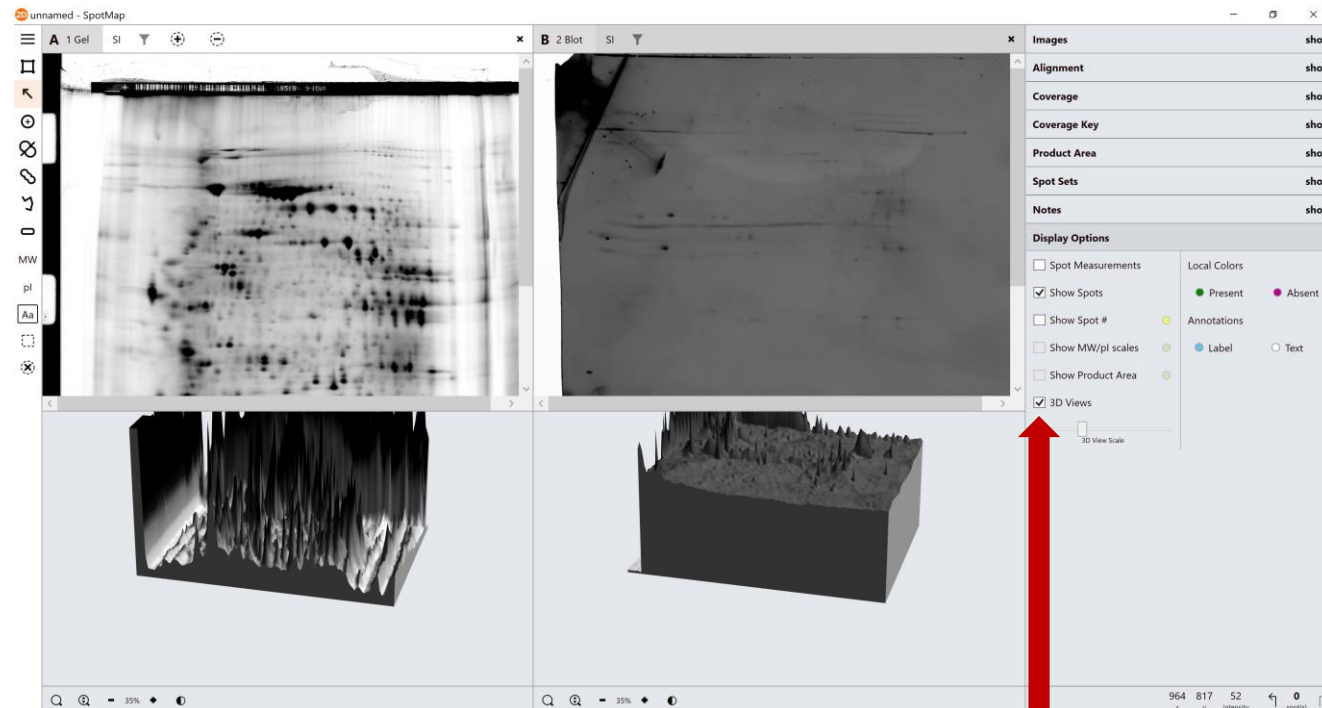
For more information, please email michael.hutchins@totallab.com

Click here to view Secondary Image display. This allows you to overlay 2 false colour images



The contrast and false colours of the primary and secondary images can be adjusted individually

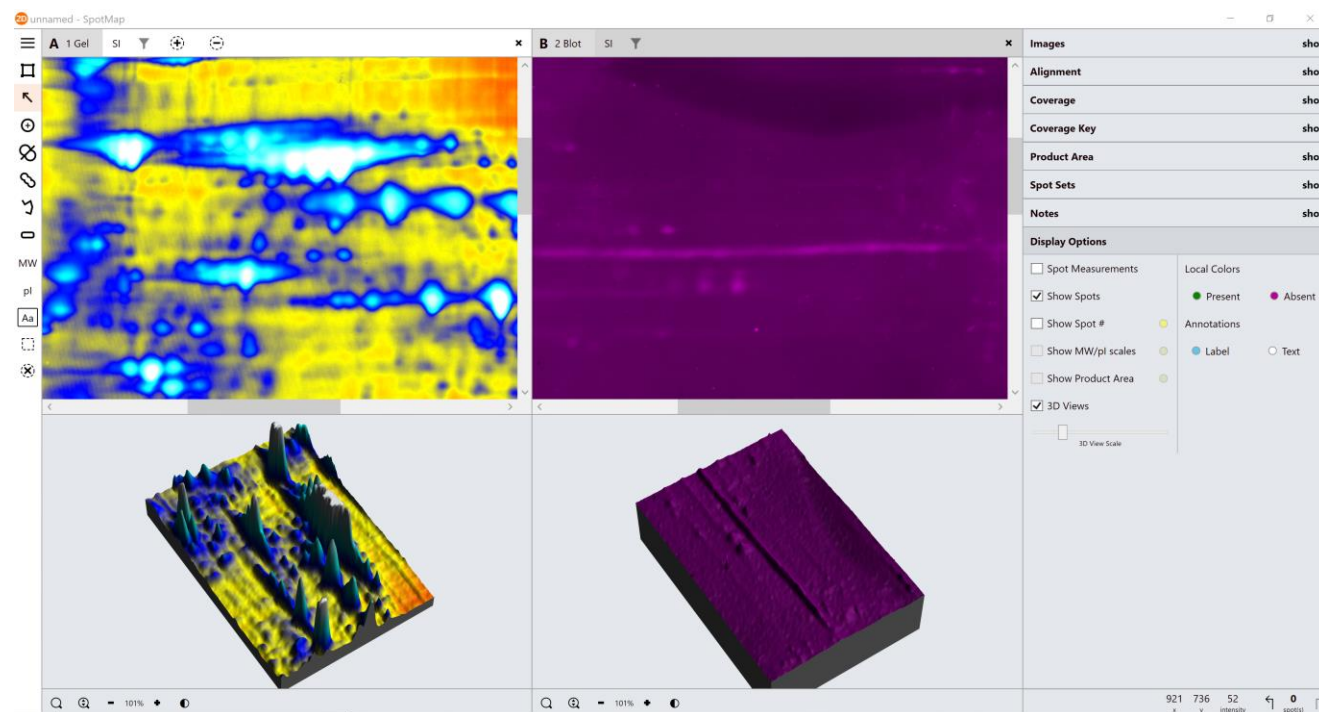
For more information, please email michael.hutchins@totallab.com



Click to open
Display Options

Check the 3D Views box
to open the 3D mode

For more information, please email michael.hutchins@totallab.com

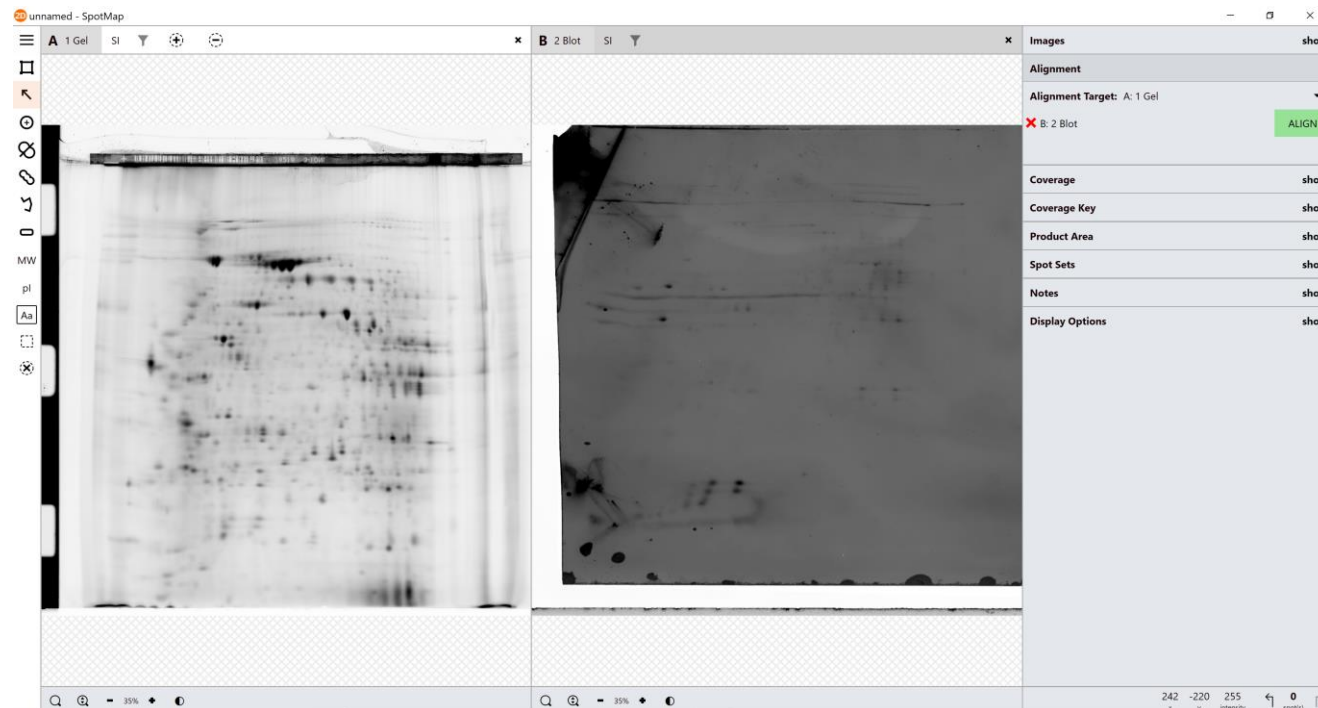


3D mode allows zooming and changes to contrast and colour as well as rotation and altering the 3D view scale using the mouse buttons and wheel

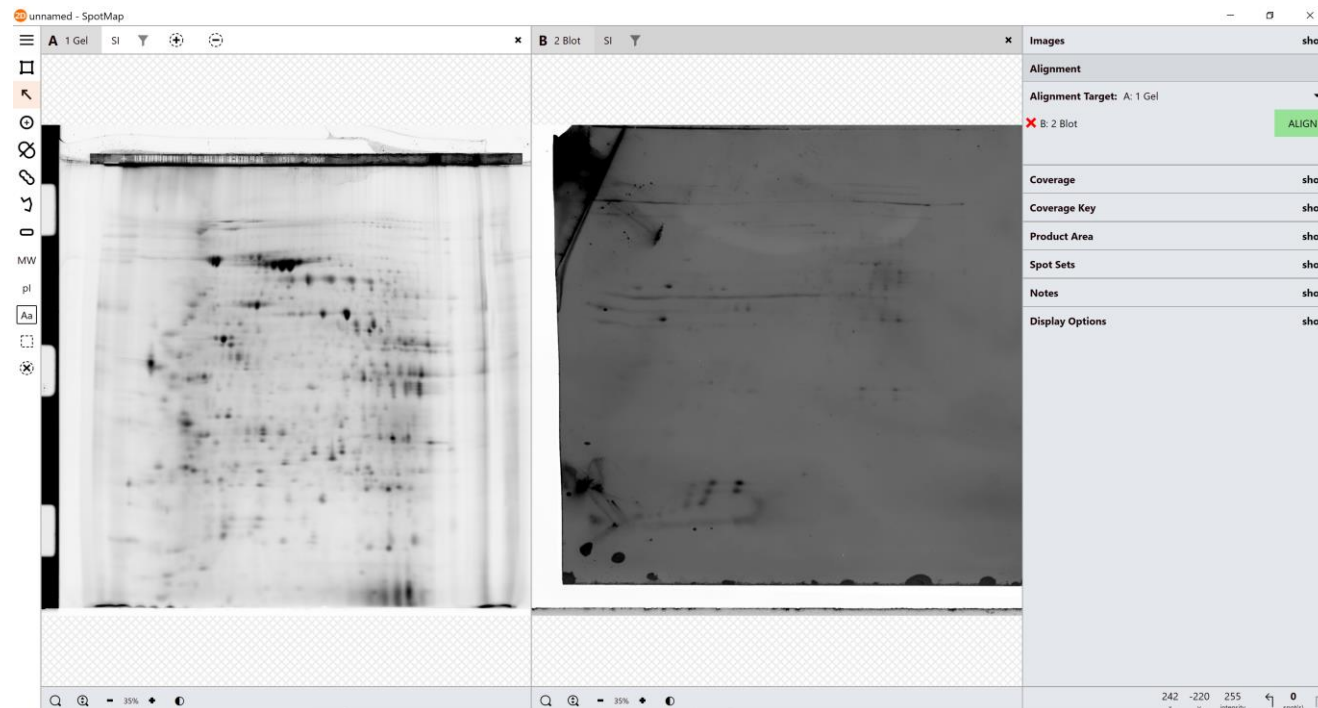
For more information, please email michael.hutchins@totallab.com

Alignment

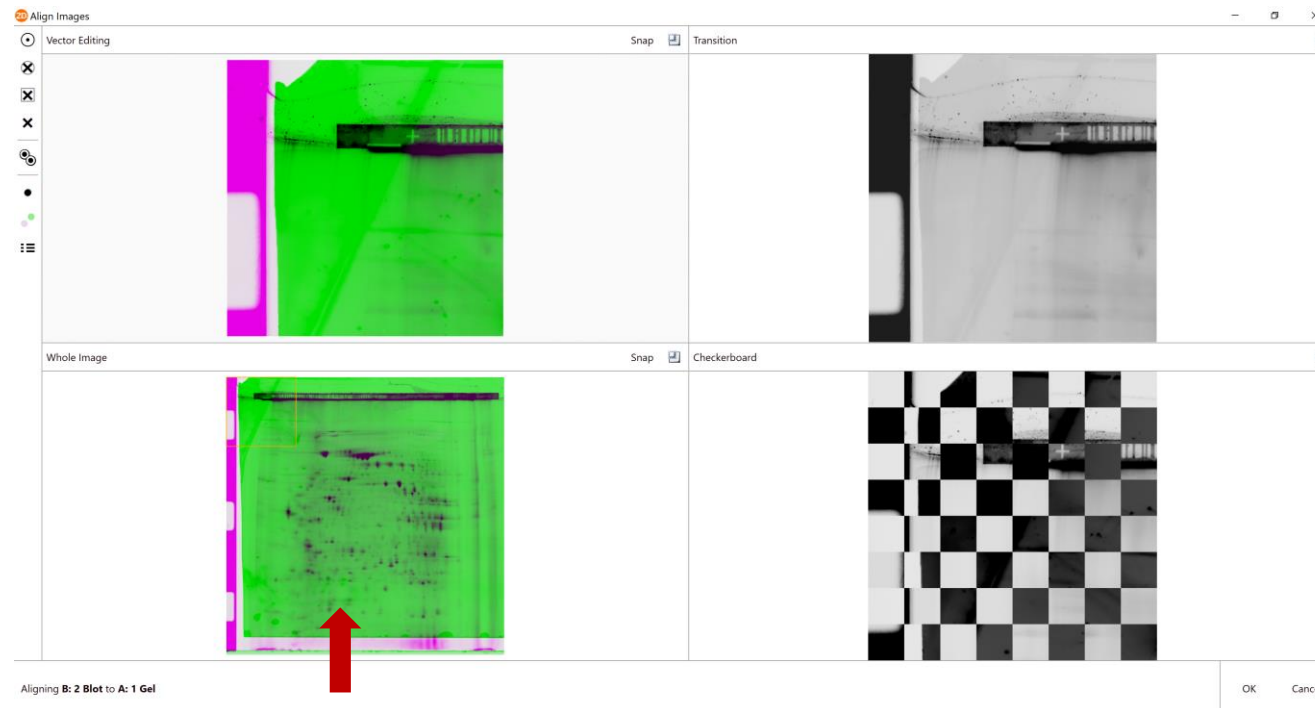
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Set A: 1 Gel to
Alignment Target



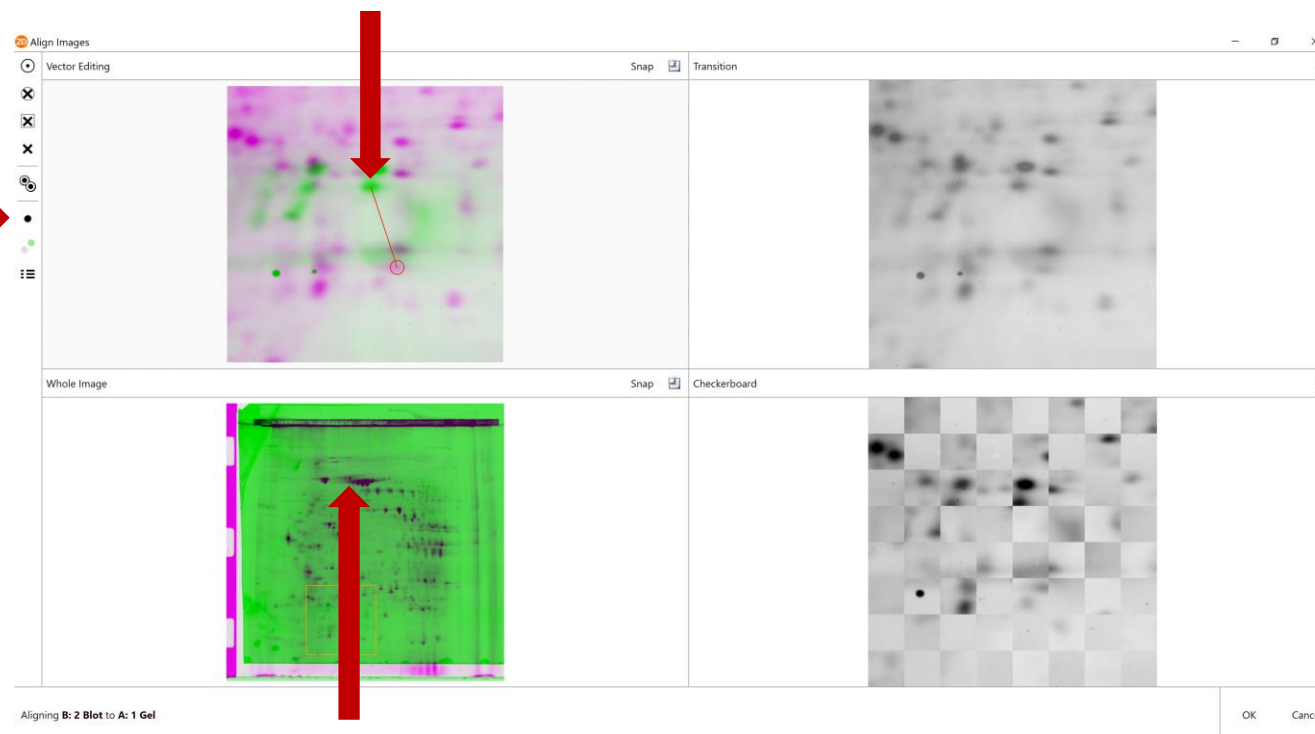
Click Align to align
the images



Click here to view this
area in more detail

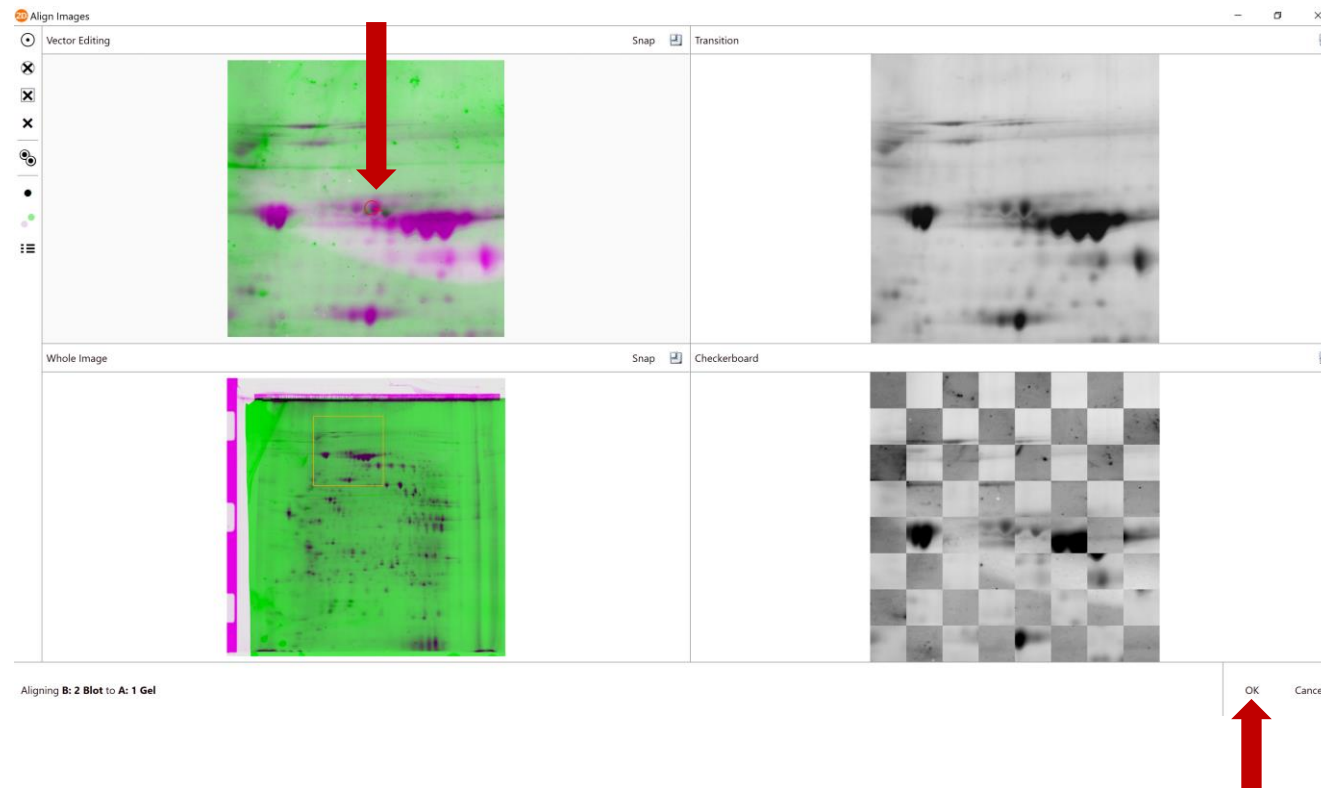
Click and drag this green spot
onto the corresponding pink
one to create a red vector

Then click
this button

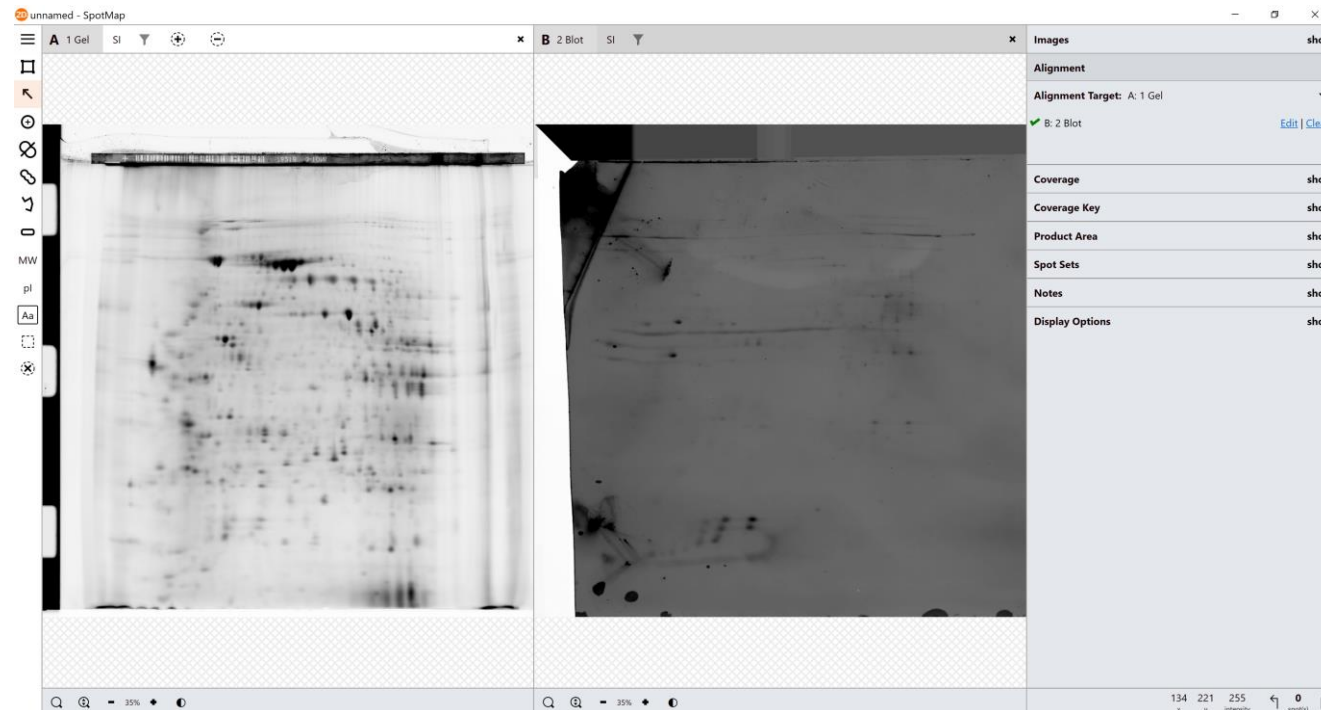


Finally, click here
to view this area

Click and drag this green spot
onto the corresponding pink
one to create a red vector

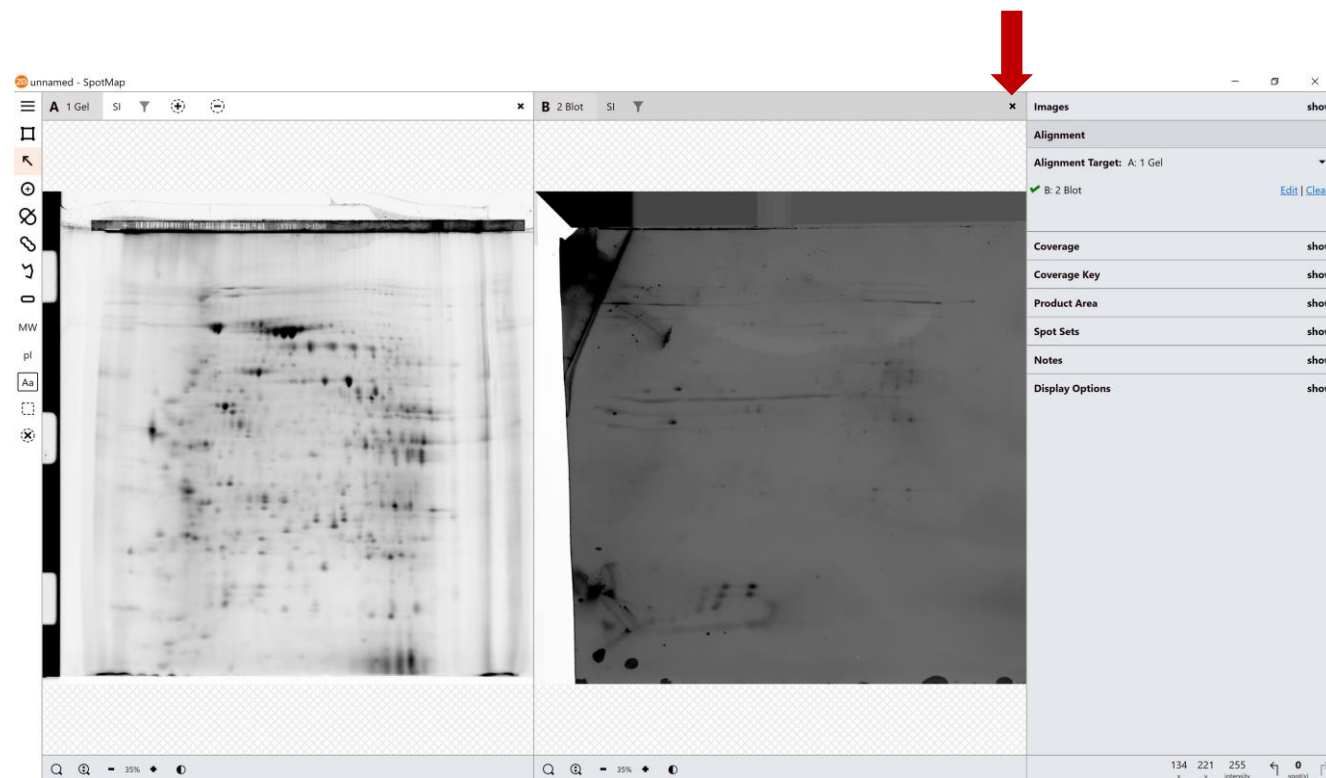


Now click OK



Alignment can be
edited or cleared
at any time

Now close the Western blot
image by clicking the cross



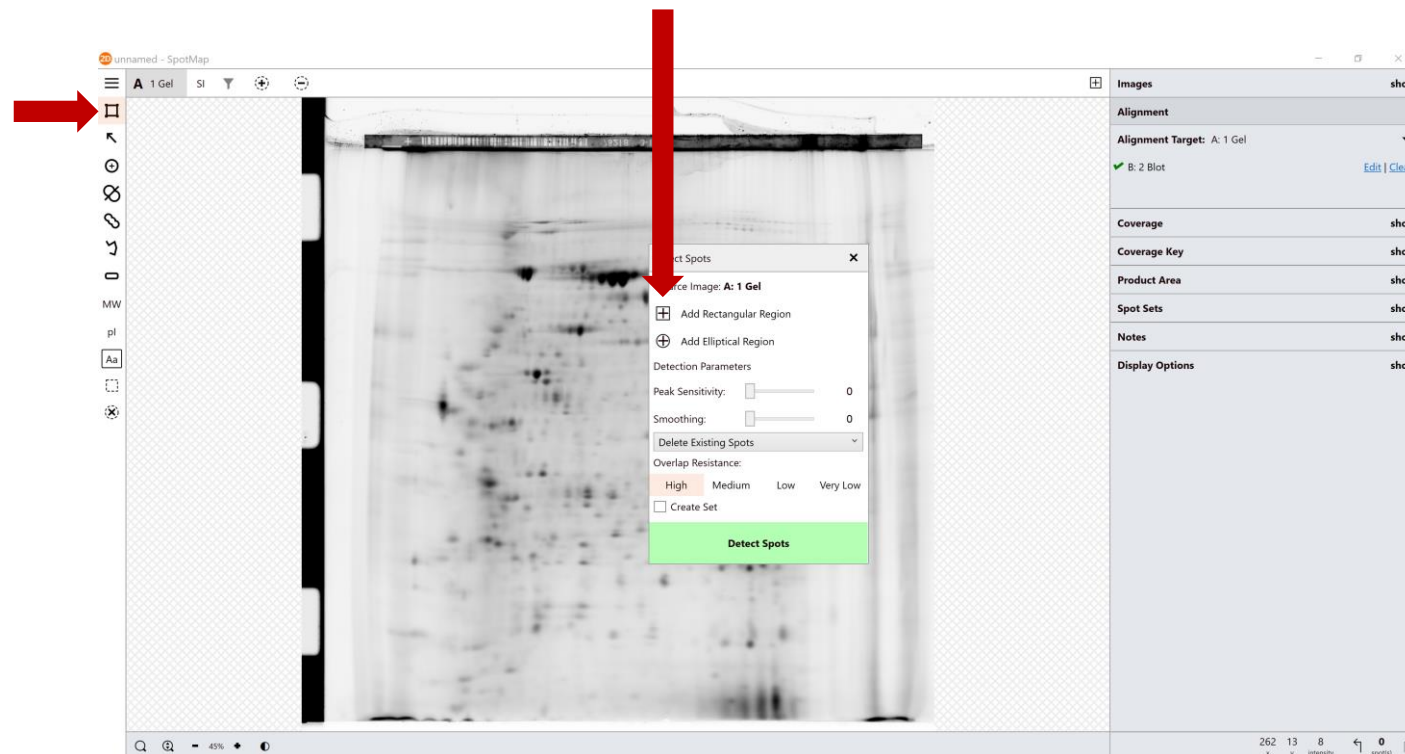
For more information, please email michael.hutchins@totallab.com

Spot Detection on Gel

For more information, please email michael.hutchins@totallab.com

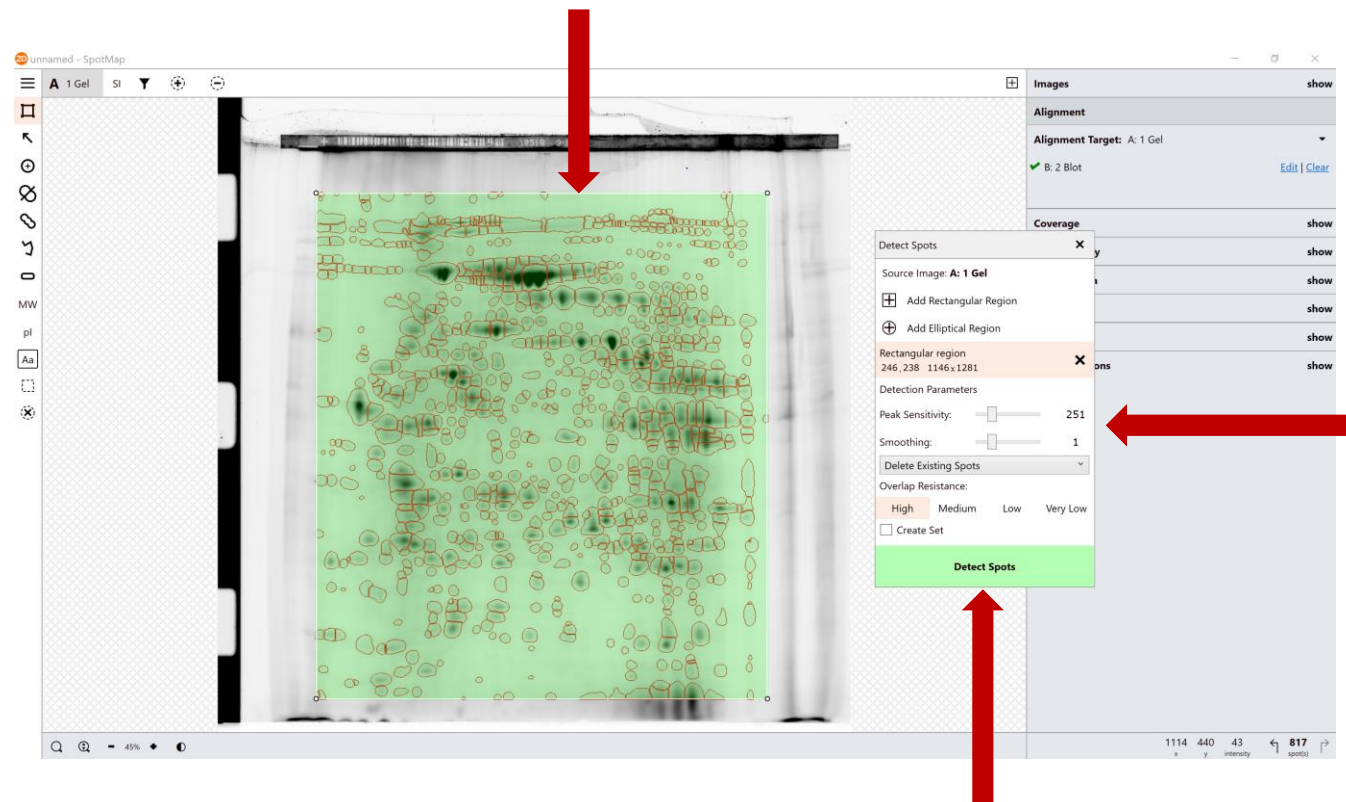
Click here to Add Rectangular
Region of interest

Click this button
to detect spots



For more information, please email michael.hutchins@totallab.com







Drag out and place region
of interest on image



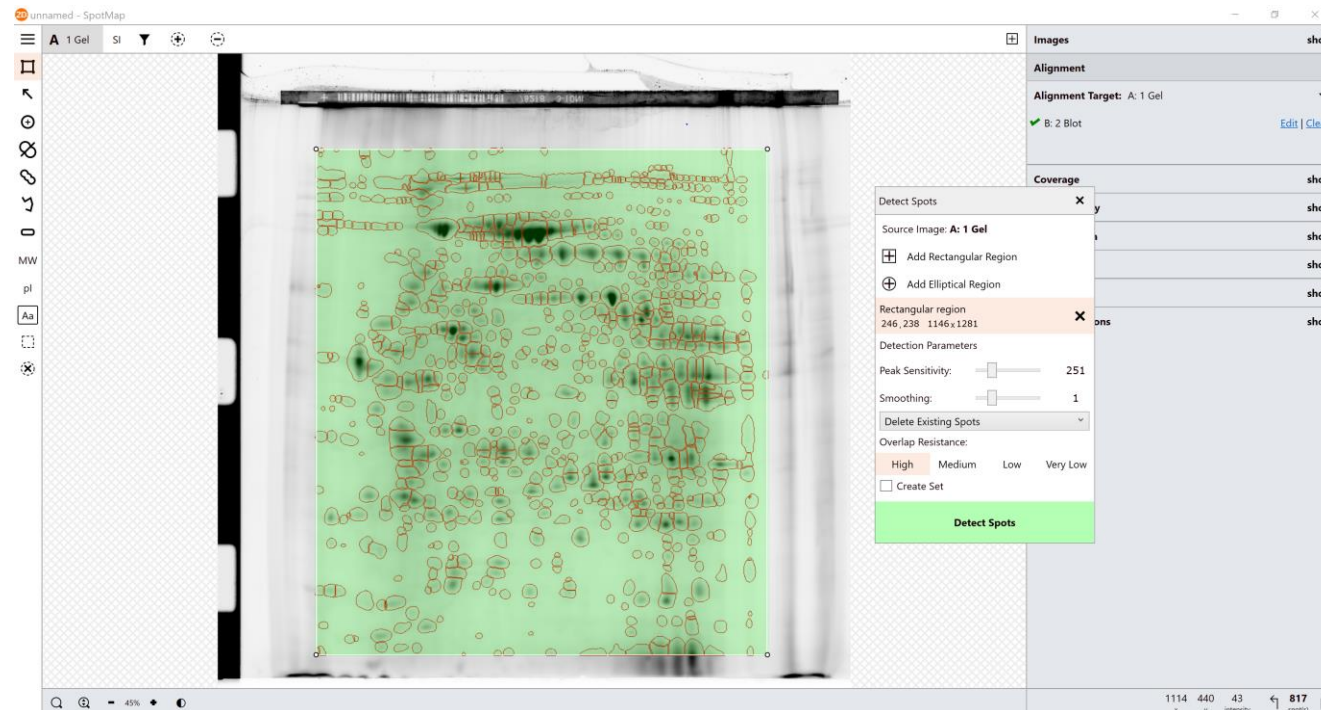
Set Peak Sensitivity to 251
and Smoothing to 1

Finally click
Detect Spots

Spots may need to be edited – on the bar on the left of the screen, you can find the following tools for editing your spot map:

-  Add tool – simply click and drag on a spot to add a new spot outline
-  Split tool – click on the peak of one spot and drag to the peak of the next to split one spot into two
-  Merge tool – click and drag across several spots to merge them into one spot
-  Freehand tool – add, split, merge and resize spots as necessary
-  Streak tool – click and drag to draw an outline around a streaked spots
- MW Add molecular weight markers to the image – create and save preset markers and drag out
- pl Add pl scale to the image – create, use and save preset pl strips and drag out
-  Add annotations as required to the image

For more information, please email michael.hutchins@totallab.com

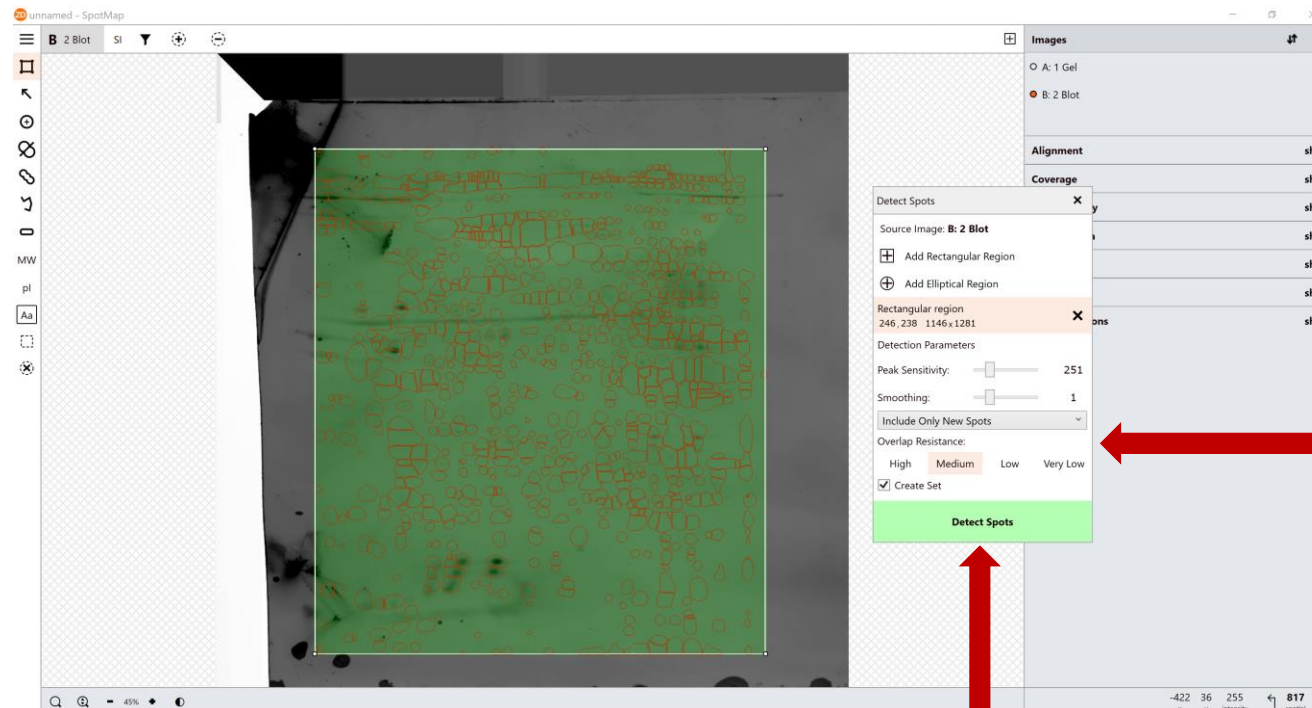


When applied, MW and pl are shown here as well as XY coordinates, spot intensity, spot numbers and the undo/redo buttons

For more information, please email michael.hutchins@totallab.com

Spot Detection on Blot

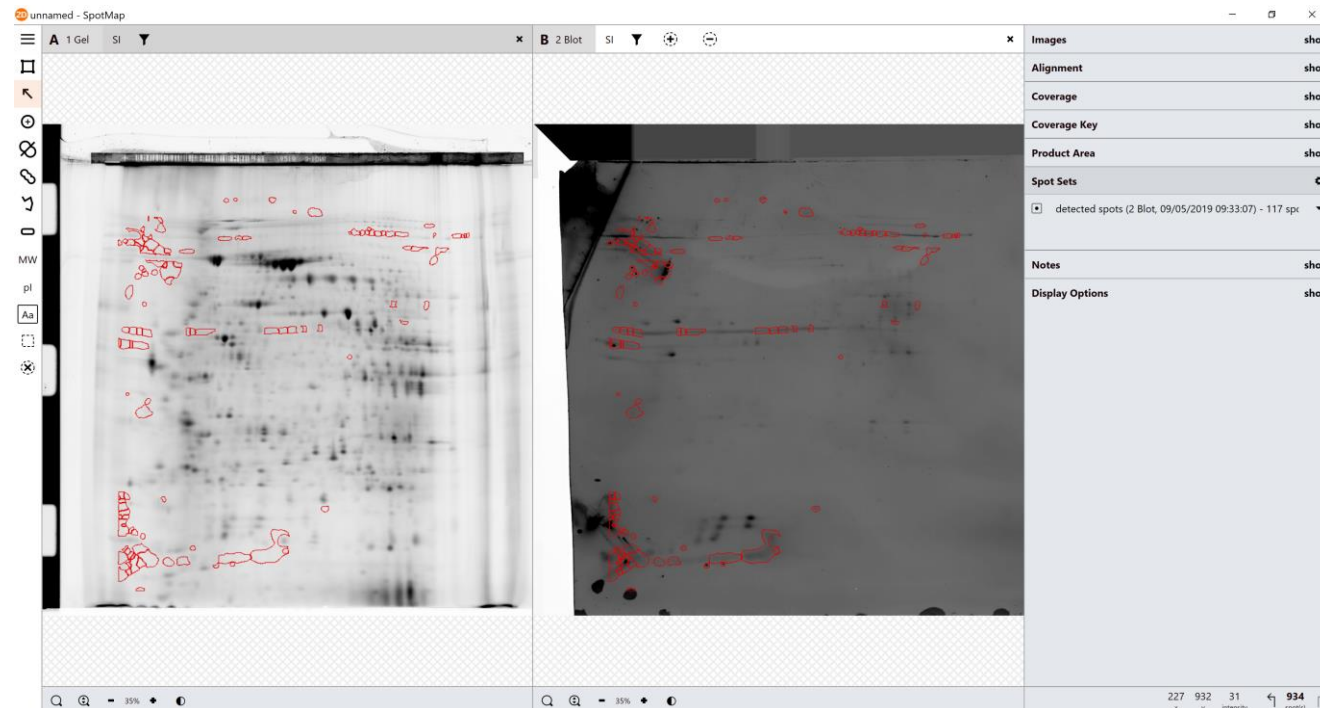
For more information, please email michael.hutchins@totallab.com



Click to select the aligned blot image

Select Include Only New Spots, Medium Overlap Resistance and tick Create Set

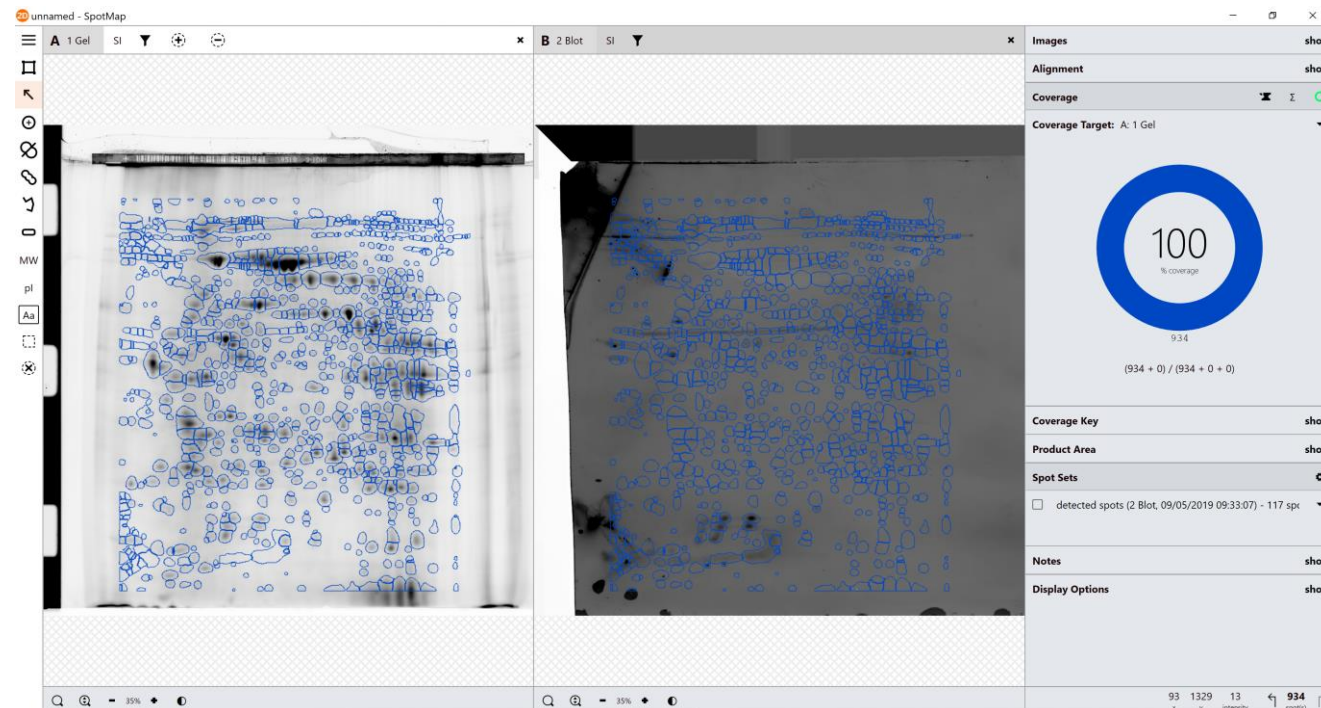
Finally click Detect Spots



Click here to view
spots detected on
blot for editing

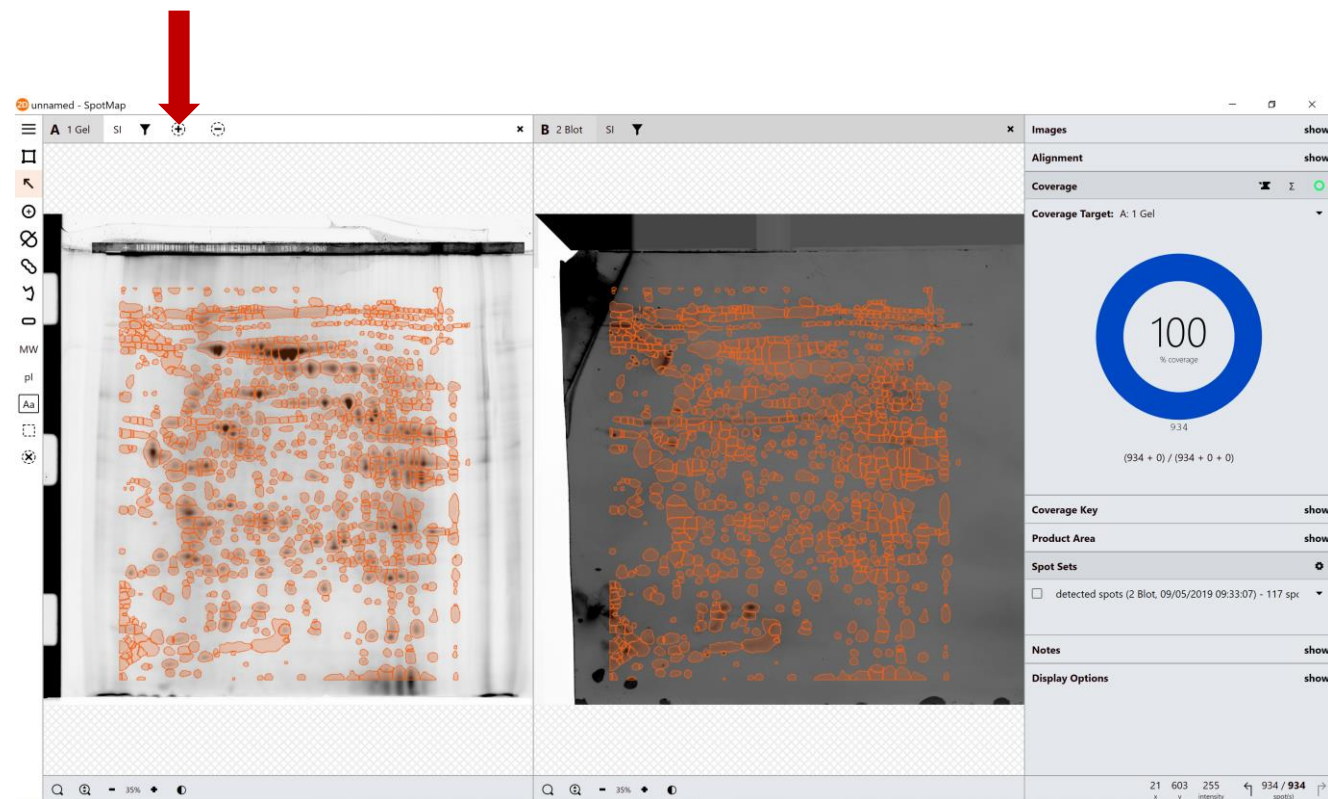
Coverage

For more information, please email michael.hutchins@totallab.com

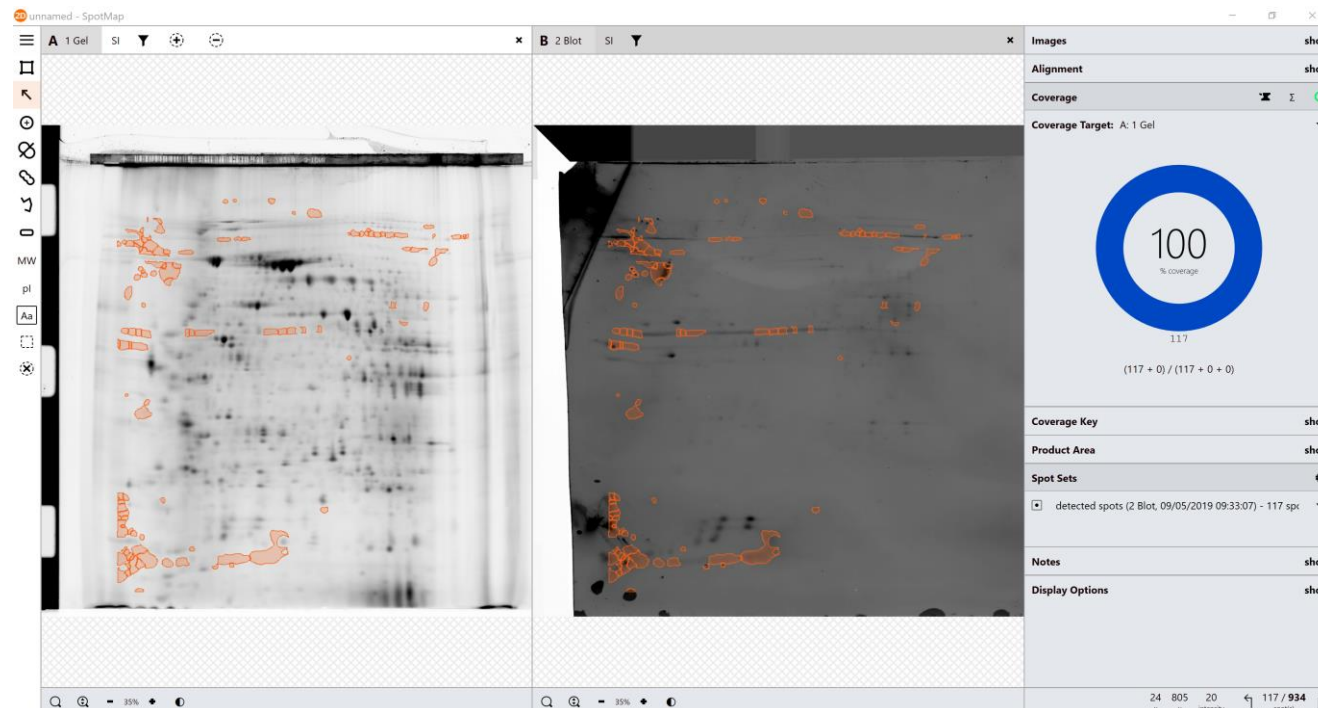


Set Coverage Target
to the Gel image

Select all the spots on
Gel Image and click
the Present button

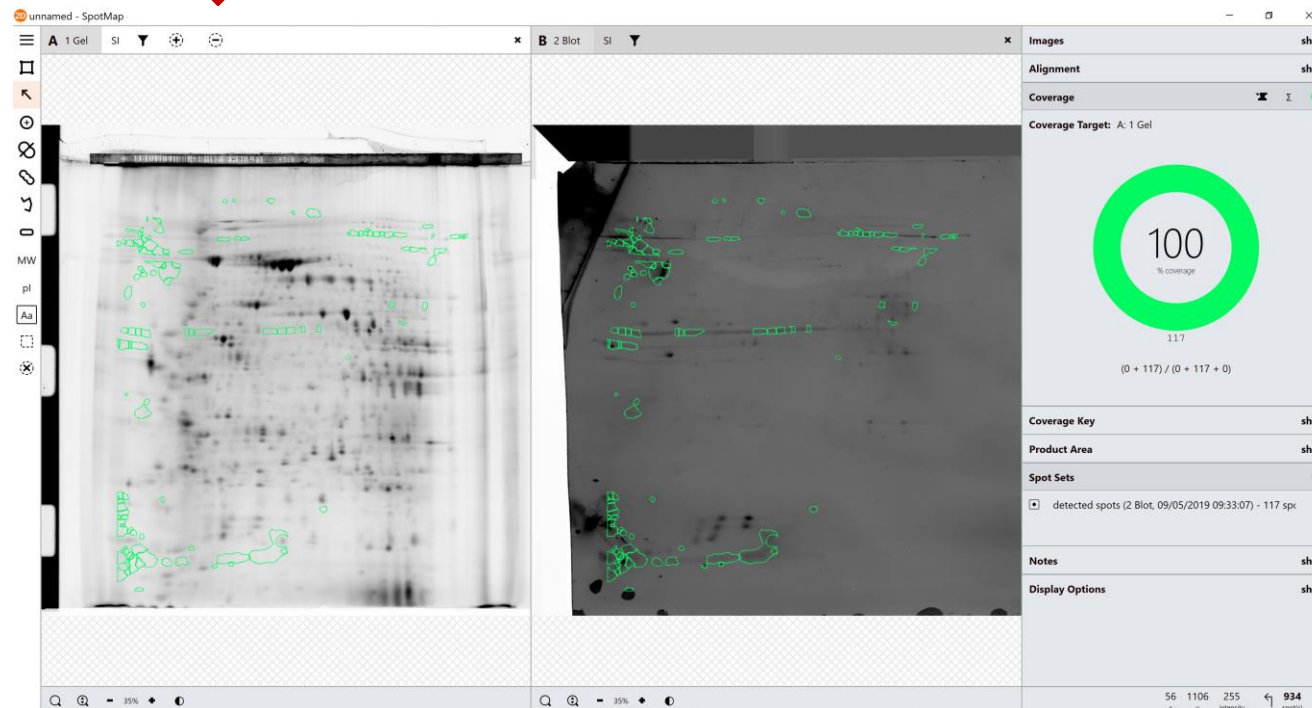


For more information, please email michael.hutchins@totallab.com



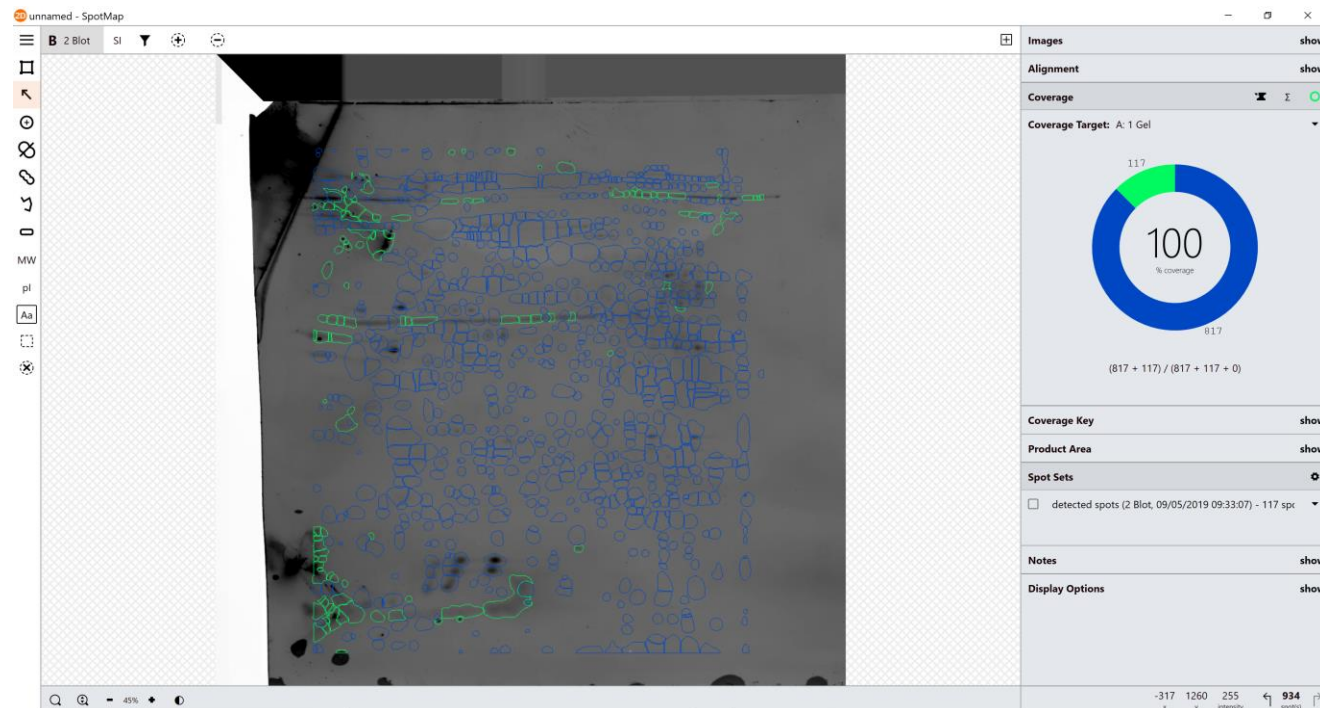
Select the spots
added to the blot

Now click the
Absent button



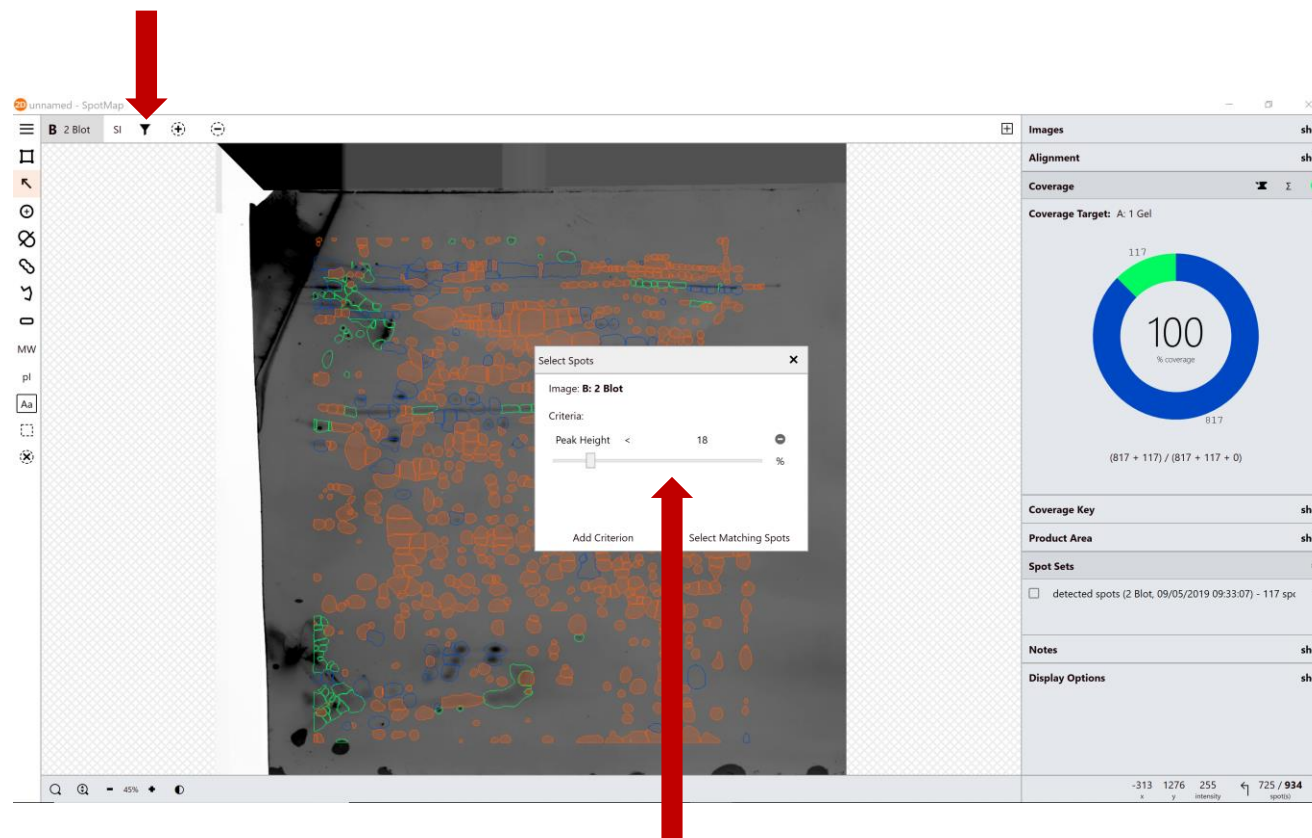
For more information, please email michael.hutchins@totallab.com

First, close the gel image by clicking the cross in the top right



Then, deselect the spots added to the blot

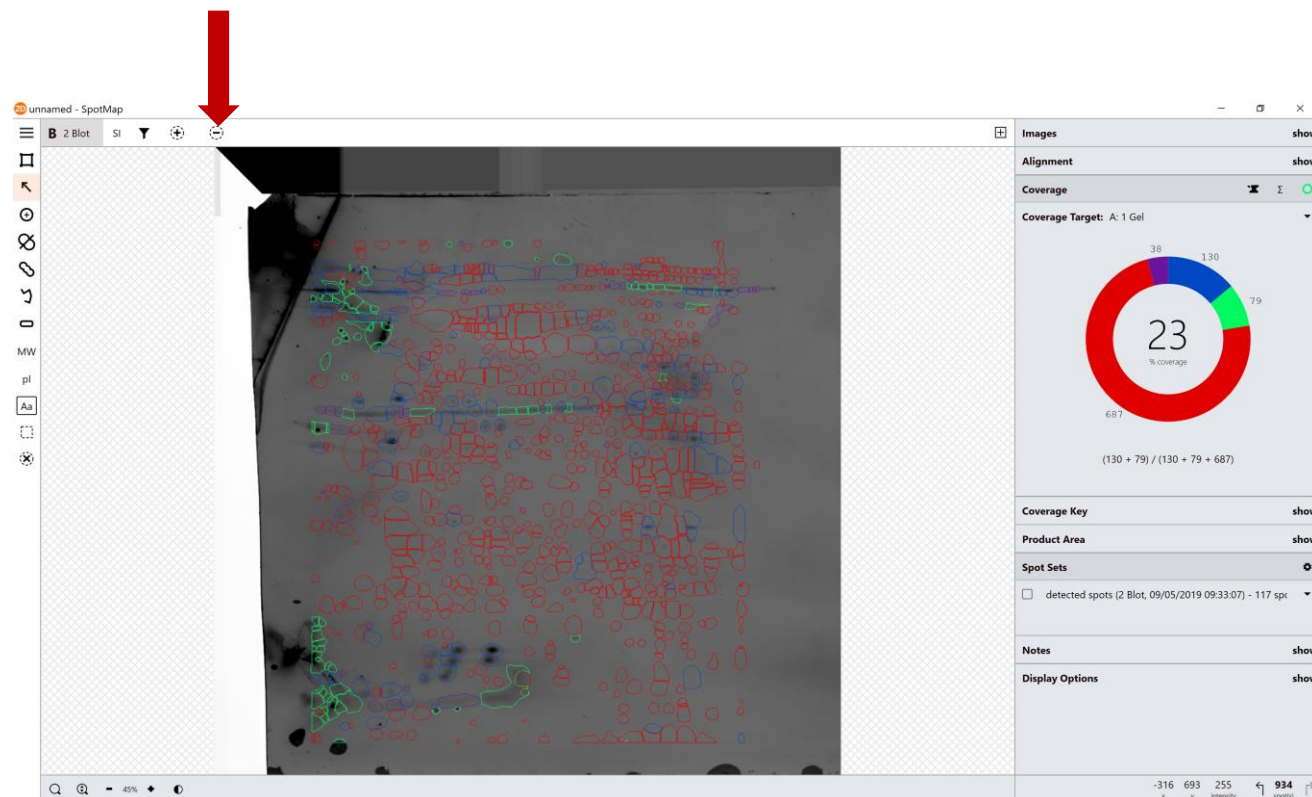
On the blot image, click
the Filter button



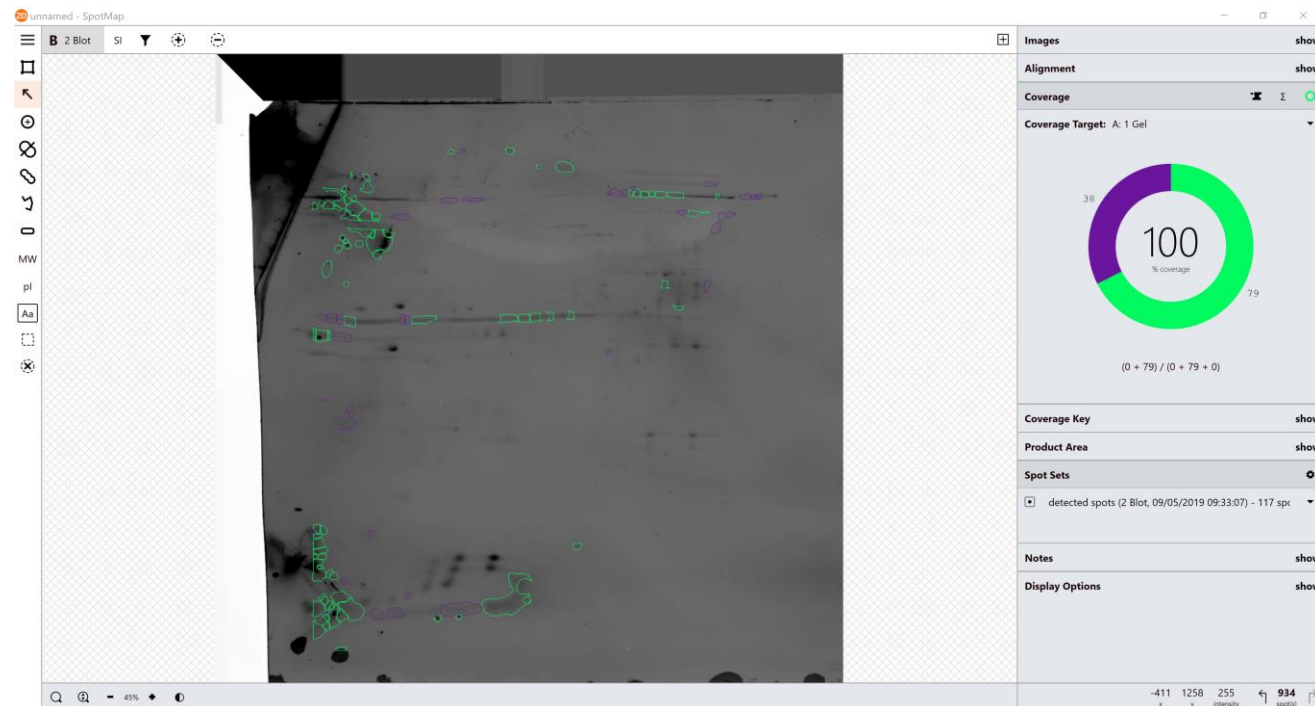
Select the Add Criterion button and
choose Peak Height. Set it to less than
18 and then Select Matching Spots

For more information, please email michael.hutchins@totallab.com

Close the Filter box and then
click the Absent button



For more information, please email michael.hutchins@totallab.com



To understand the values, open the Coverage Key

Coverage Key:

| Coverage Key | | |
|--------------------|--------|---------|
| ● Common spots | ✓ | ✓ |
| ● Missing spots | ✓ | × |
| ● Additional spots | × | ✓ |
| ● Absent spots | × | × |
| | Target | Current |

130 Common Spots – Present on the gel and the blot

687 Missing Spots – Present only on the gel

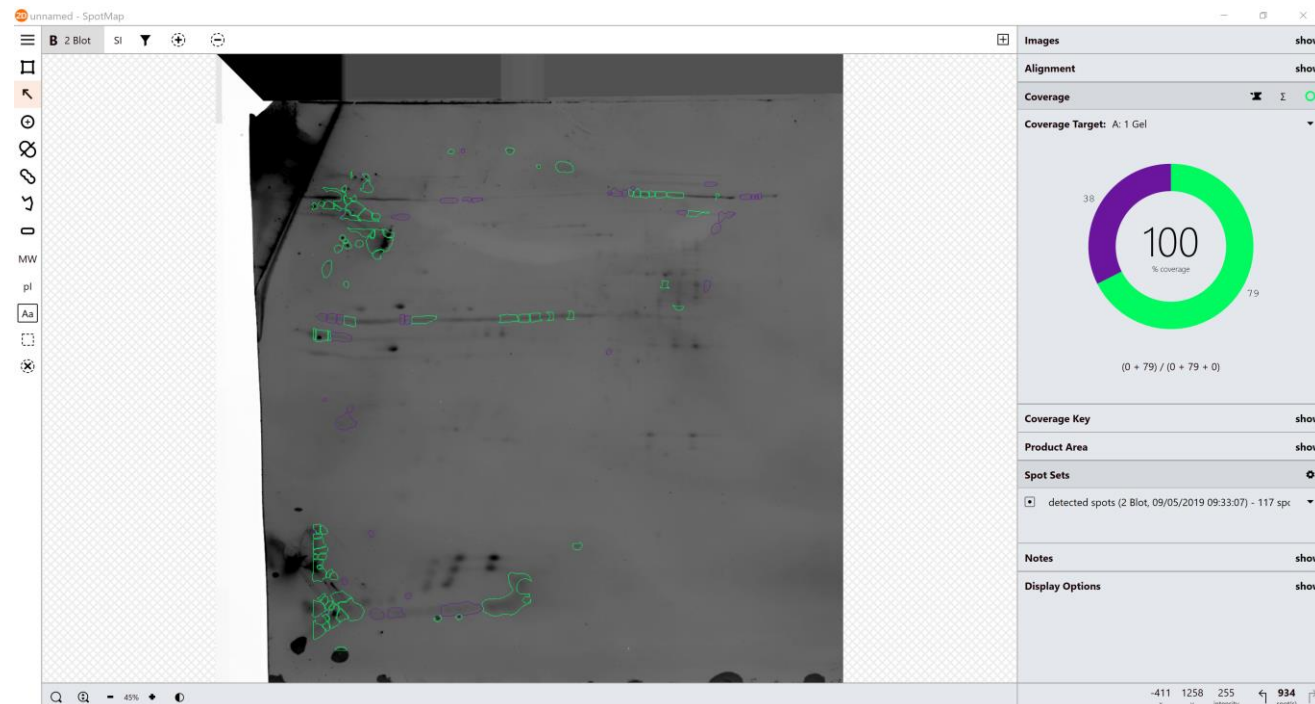
79 Additional Spots – Present only on the blot

38 Absent Spots – Not present on either image

Removing Absent Spots:

Absent Spots are not present on either image.

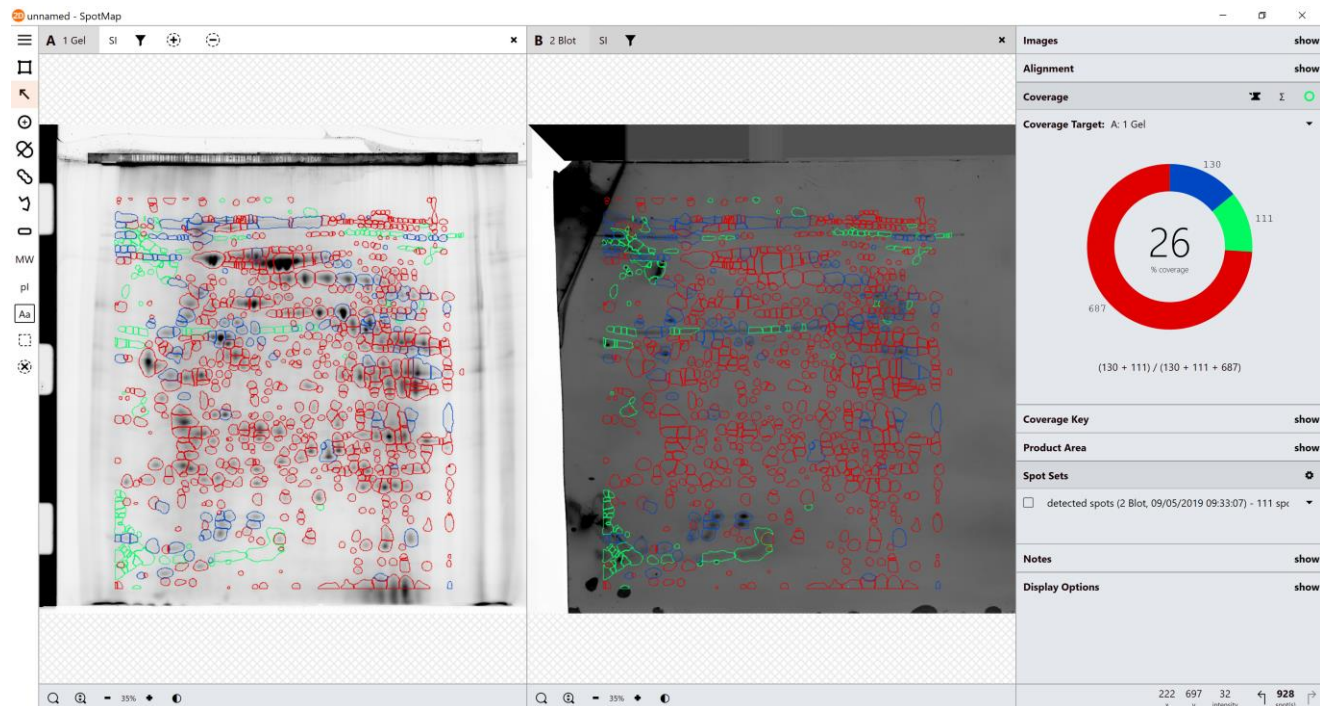
These 38 spots should be reviewed manually and then changed to present or simply deleted.



Open the Spots Sets tab and select only this group. Delete the absent (purple) spots.

Once completed, deselect the Spot Set to view all spots.

Open the gel image and you will be able to view the complete coverage map across both images.

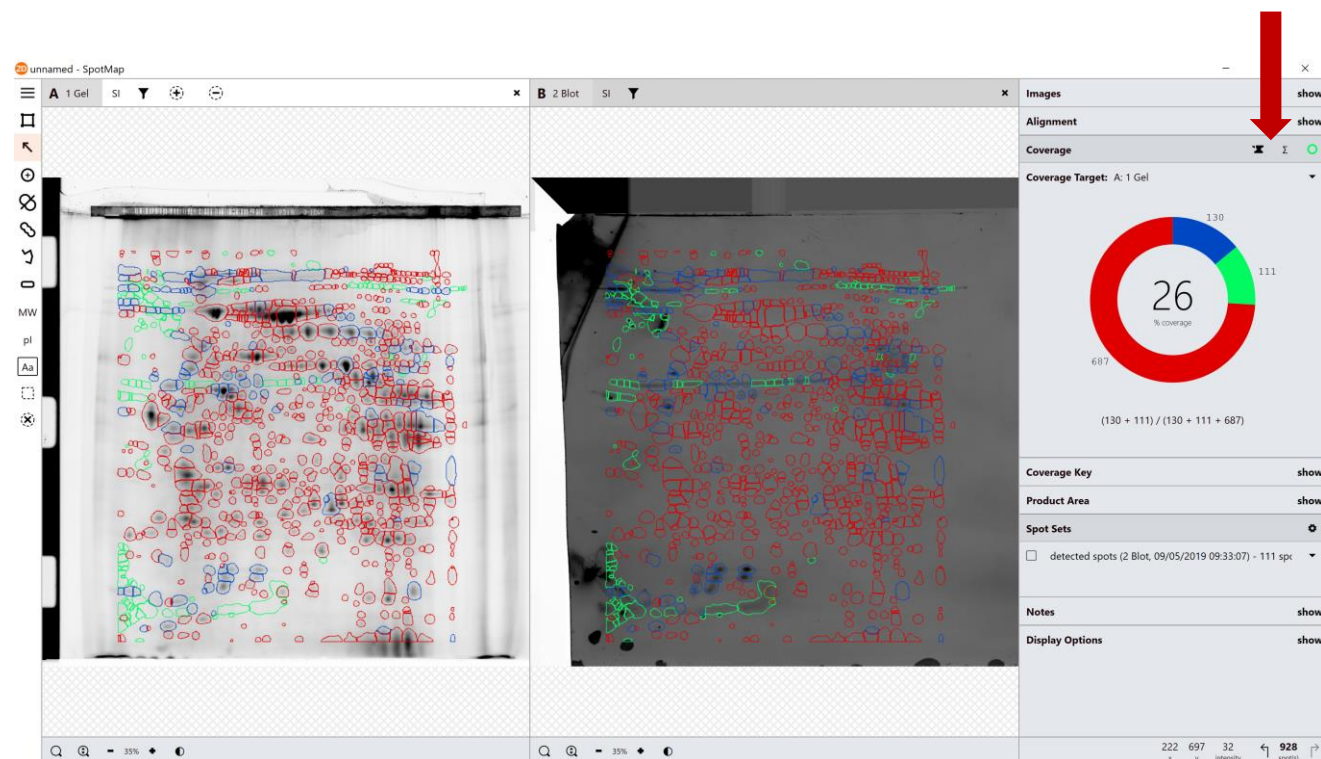


Coverage Equations, Coverage by Volume & Quadrants

For more information, please email michael.hutchins@totallab.com

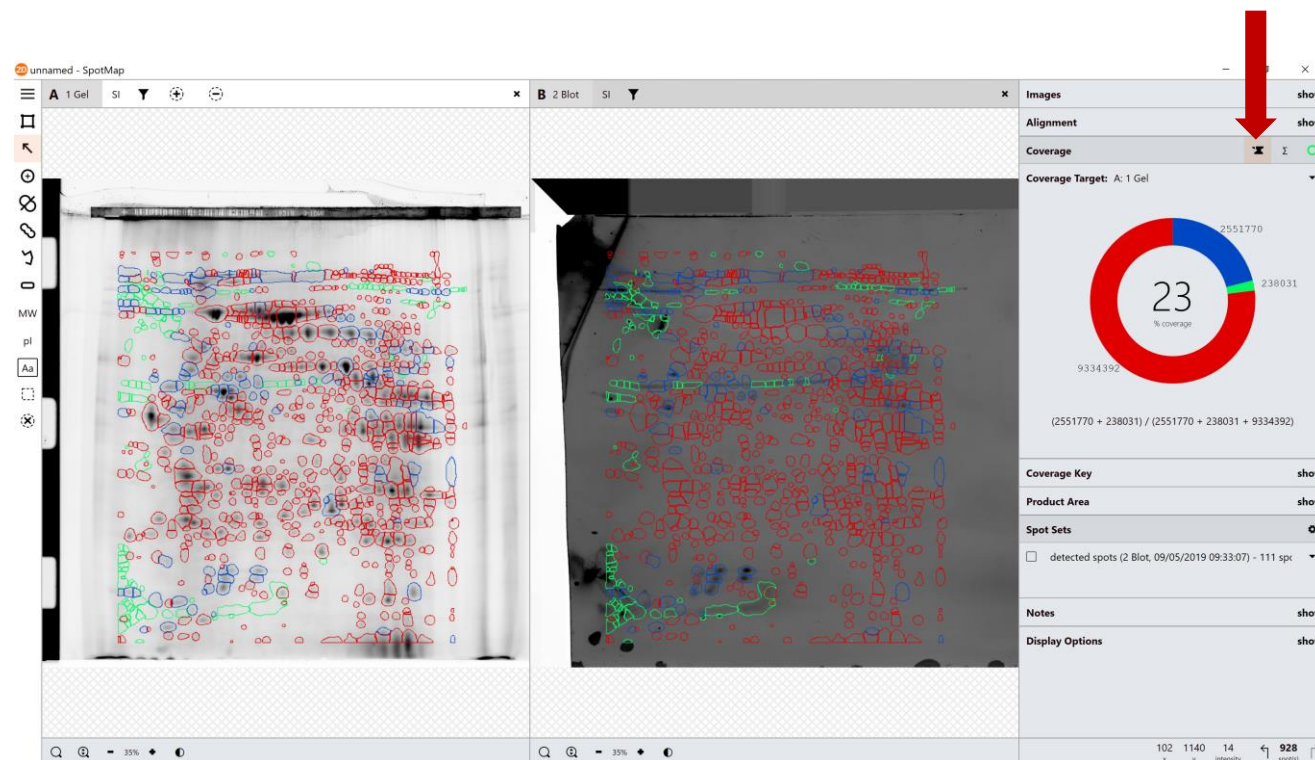
These 2 buttons allow you to:

- View Coverage by Volume
- View Different Coverage Equations



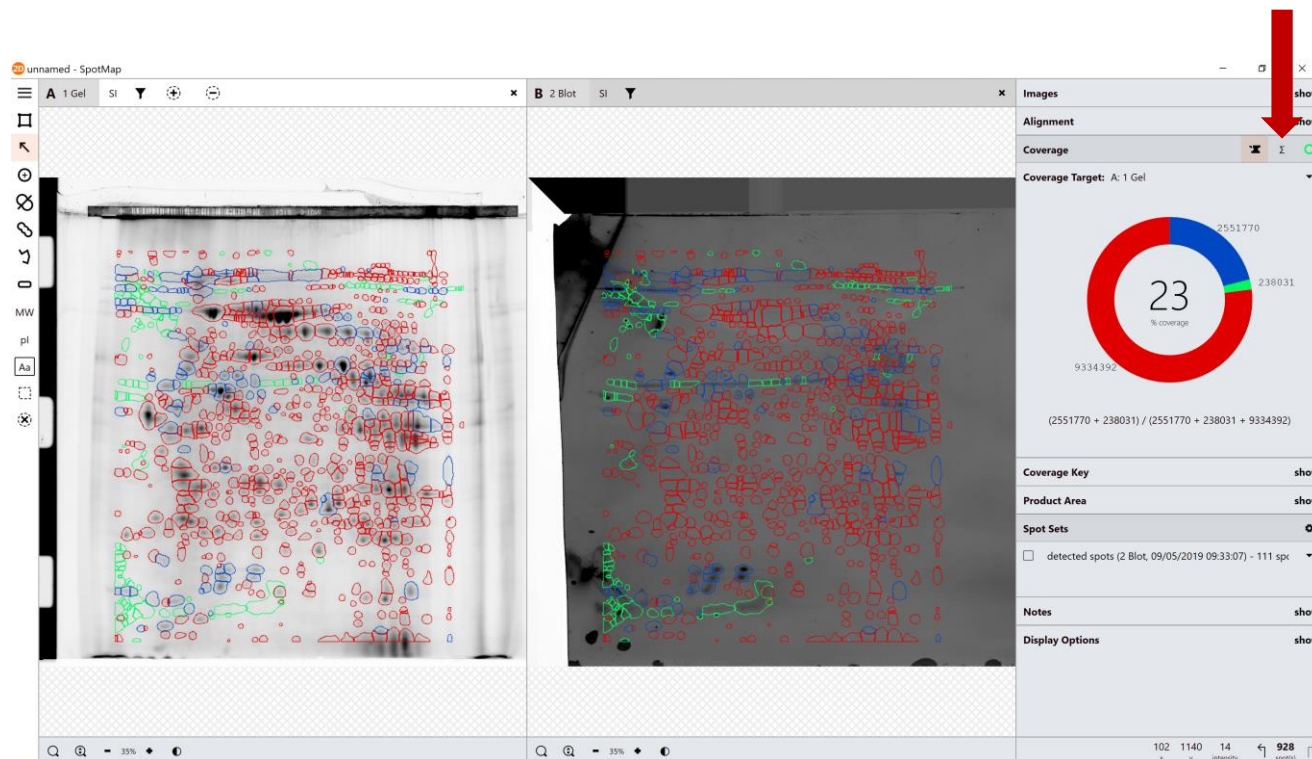
For more information, please email michael.hutchins@totallab.com

Click this button to view
Coverage by Volume. This
determines percentage protein
abundance in common spots.

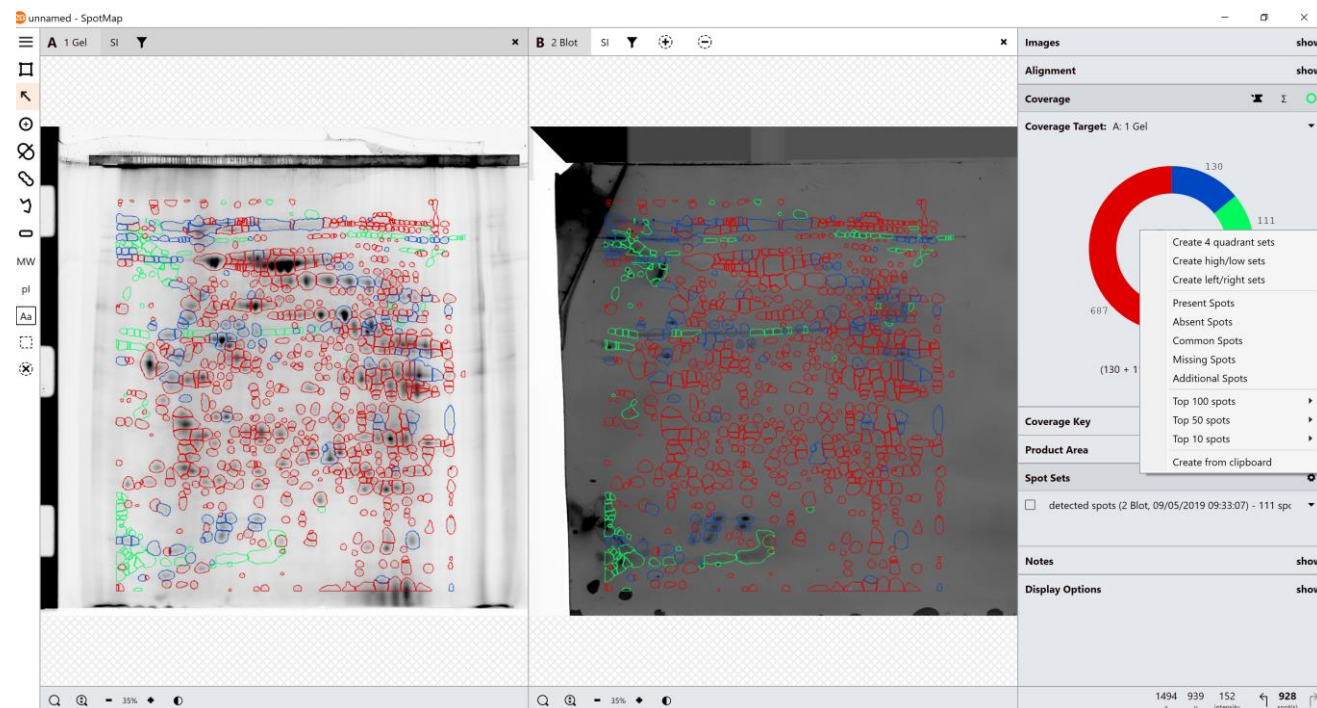


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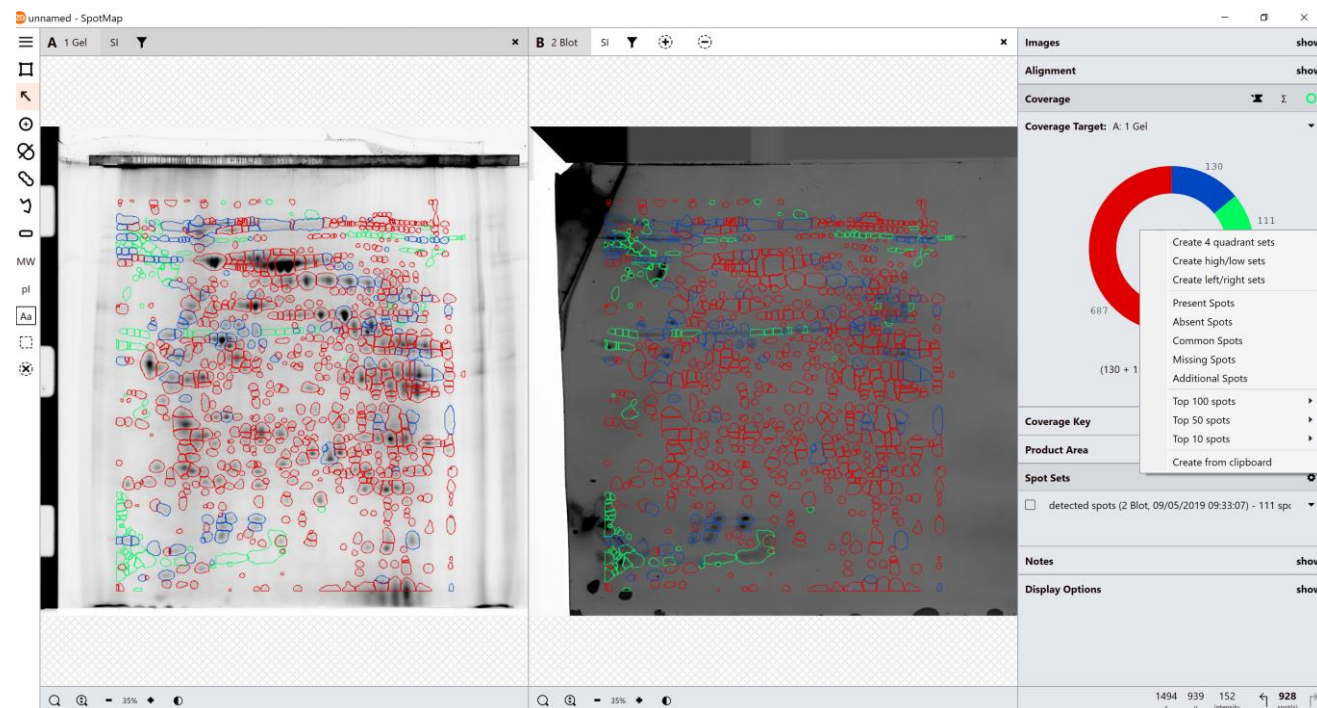
Click this button to view different coverage equation options. Different equations can give varying coverage values.



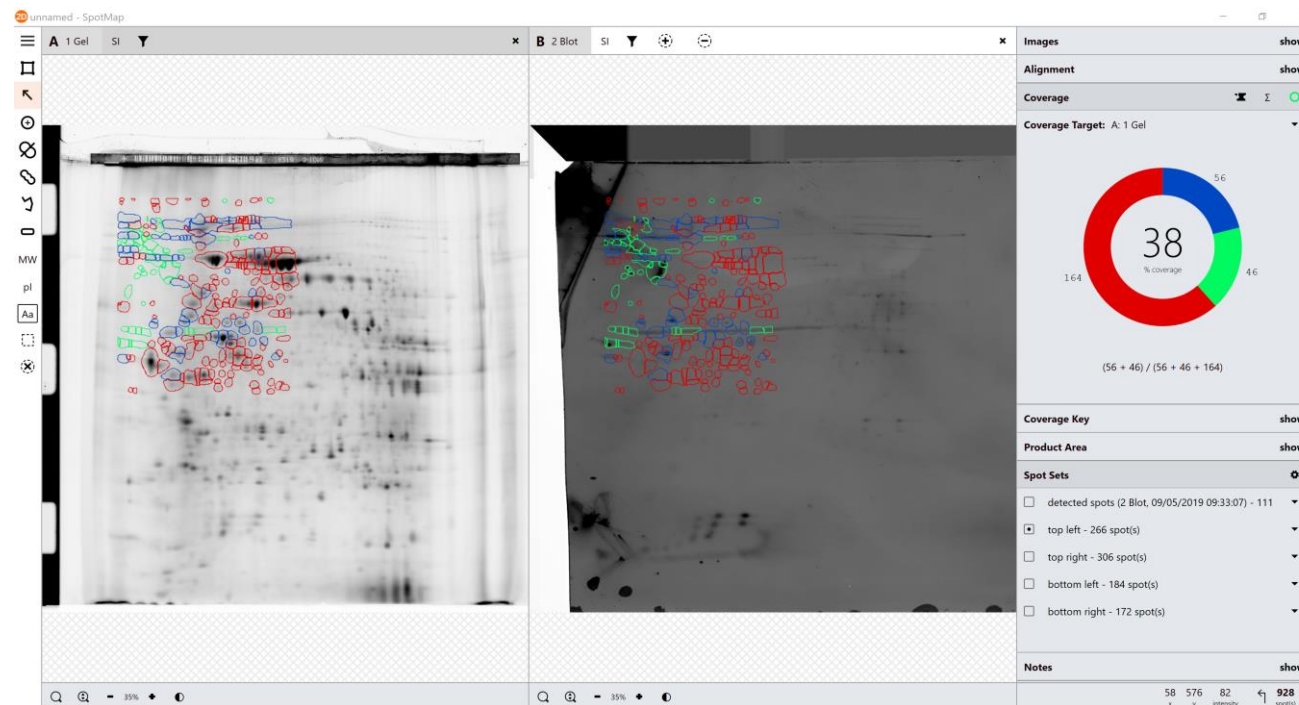
For more information, please email michael.hutchins@totallab.com



Open the Spots Sets tab
and click the Cog button



Now select Create
4 quadrant sets

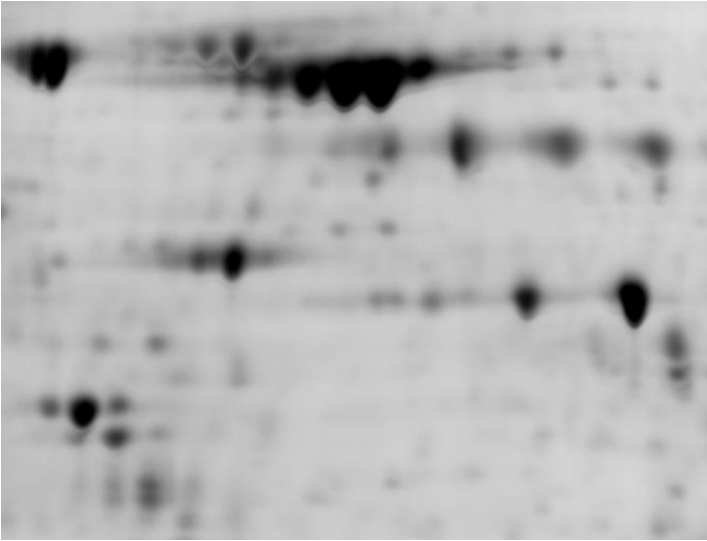


You can now view the
Coverage values by
different quadrants

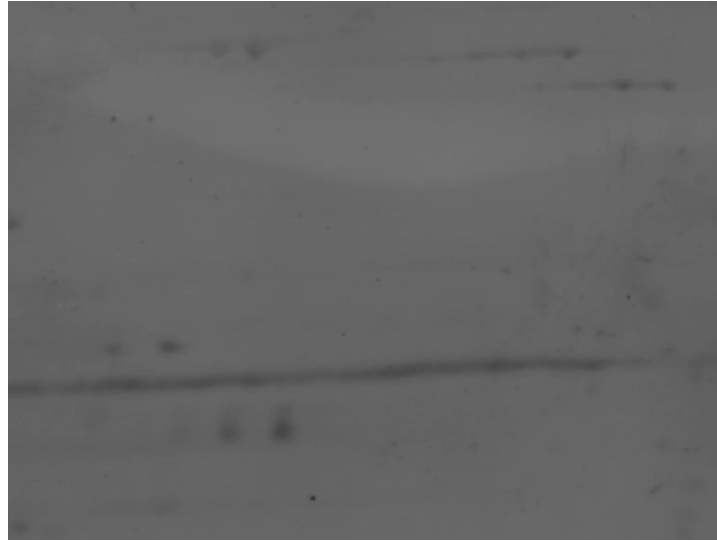
Product Spots

For more information, please email michael.hutchins@totallab.com

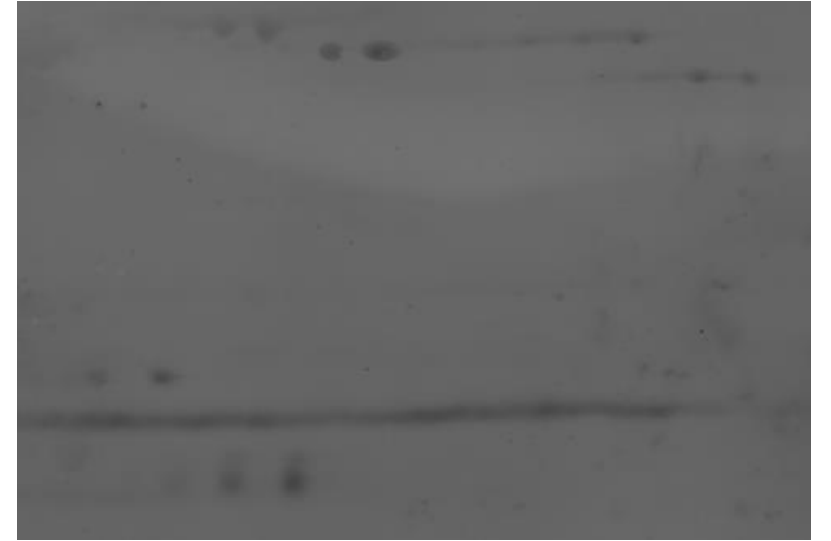
Required images:



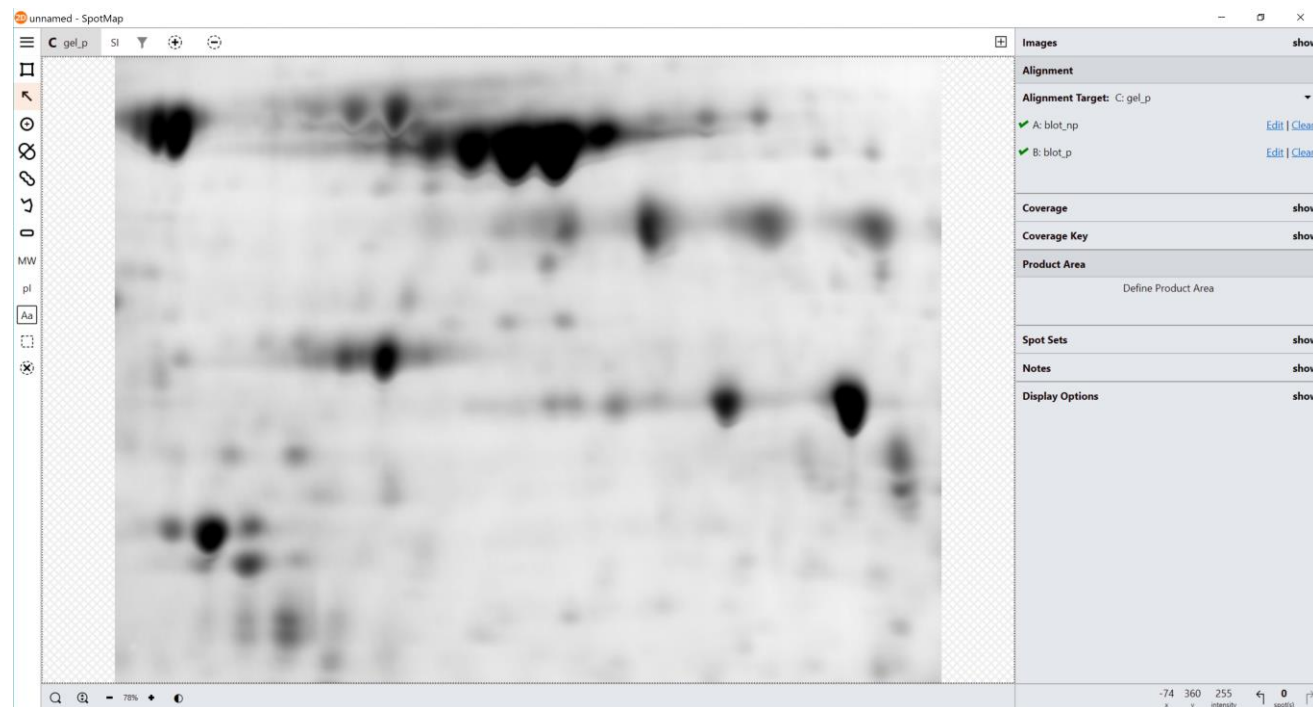
(1) Gel with product spots



(2) Blot with no product spots

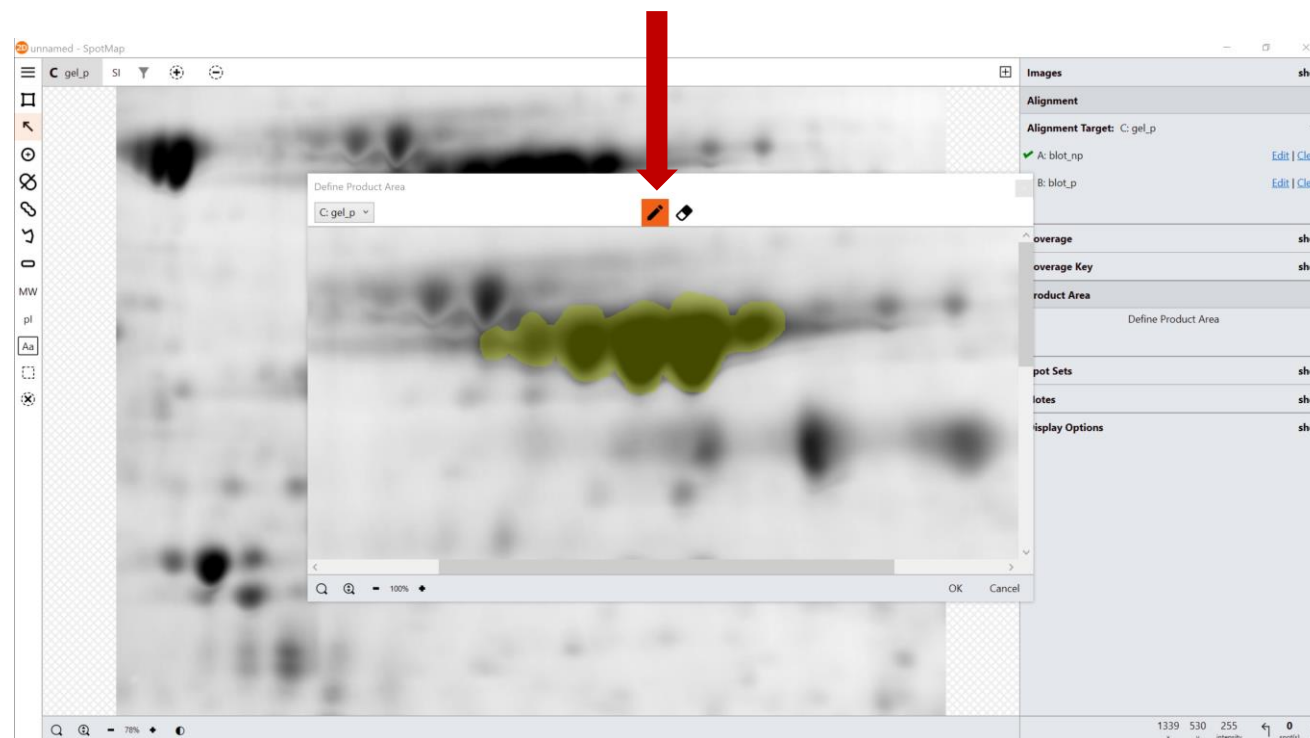


(3) Blot with product spots

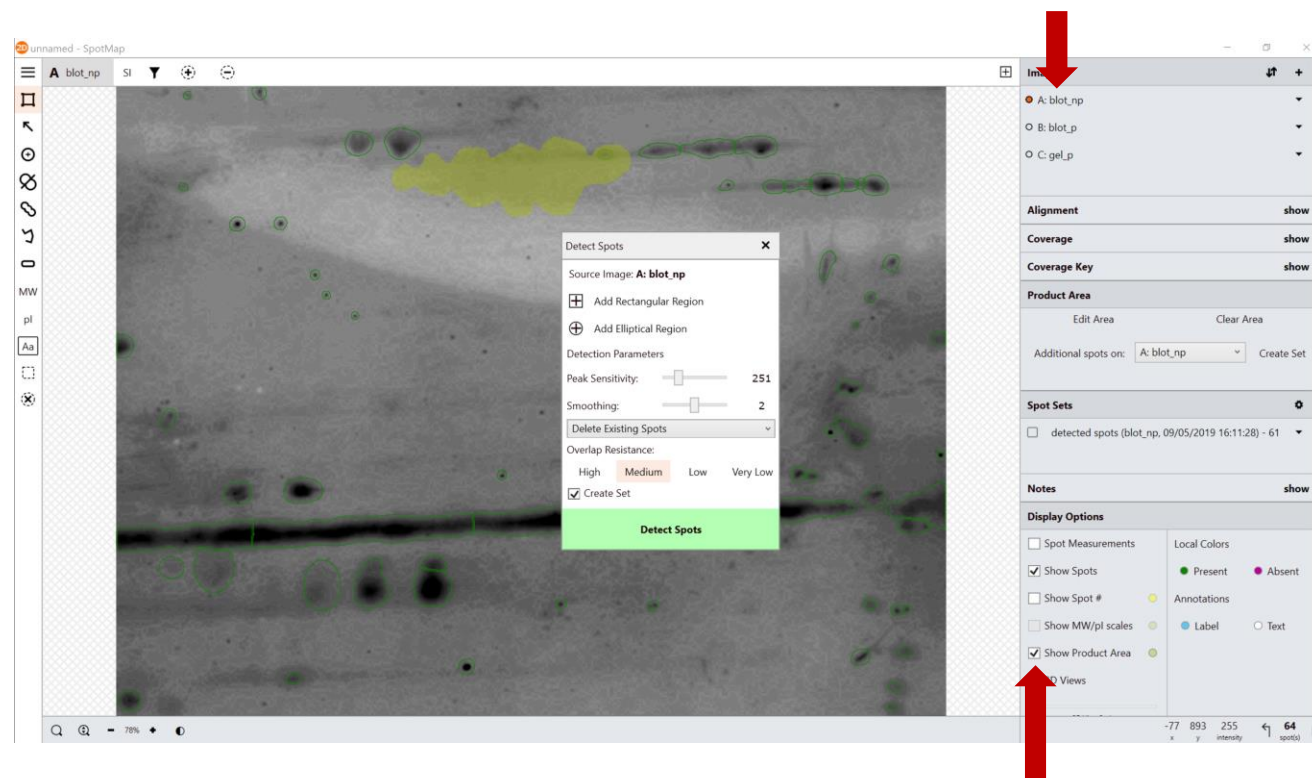


Open Alignment and Product Area tabs. Set Alignment Target to Gel image (with product spots). Click Define Product Area

Use Pen tool to draw around product spots. This will create a highlighted mask of the area.

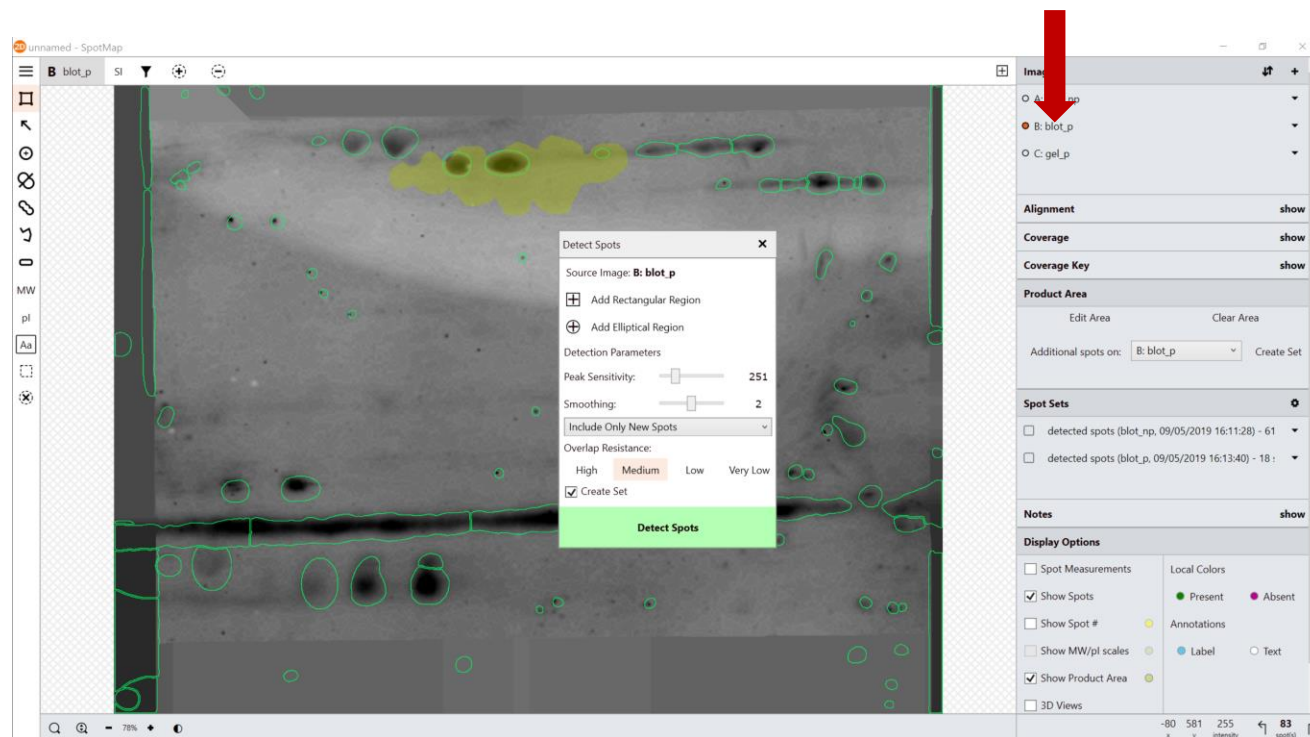


Open the Blot image (without product) and detect spots.

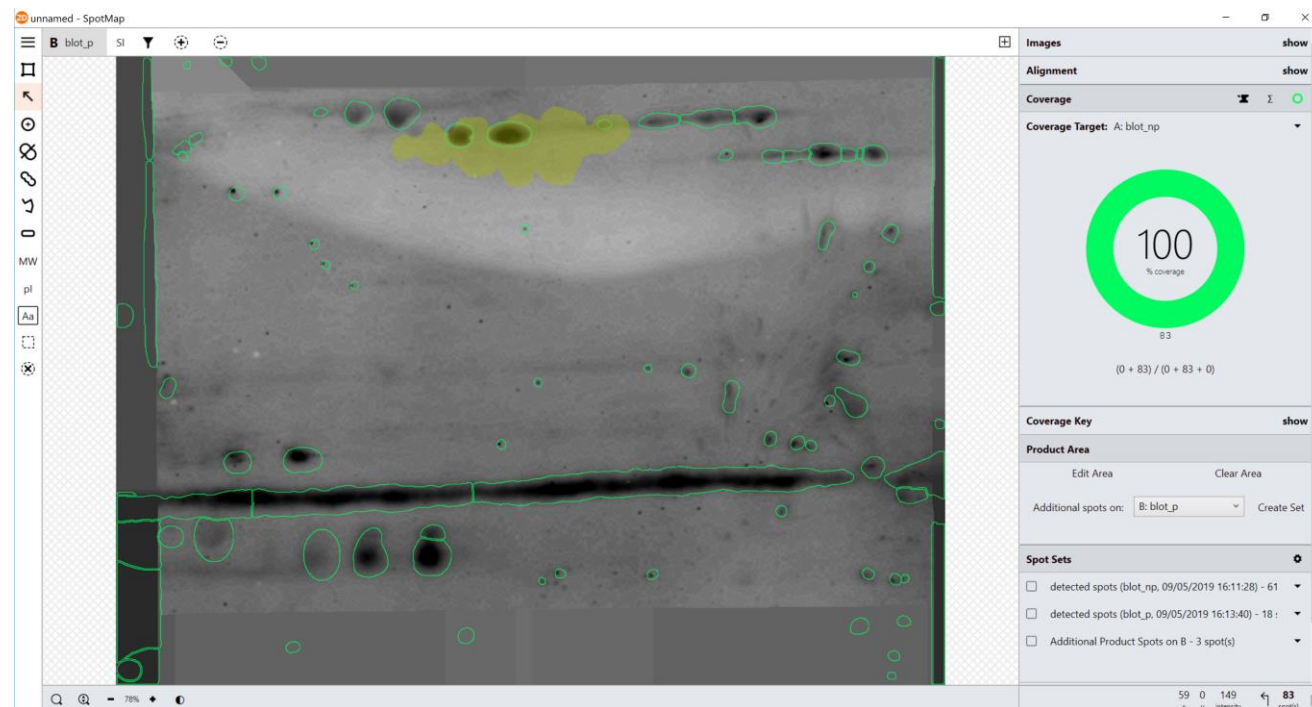


Check the Show Product Area box in Display options.

Open the Blot image (with product)
and detect spots. Make sure that
you Include Only New Spots.

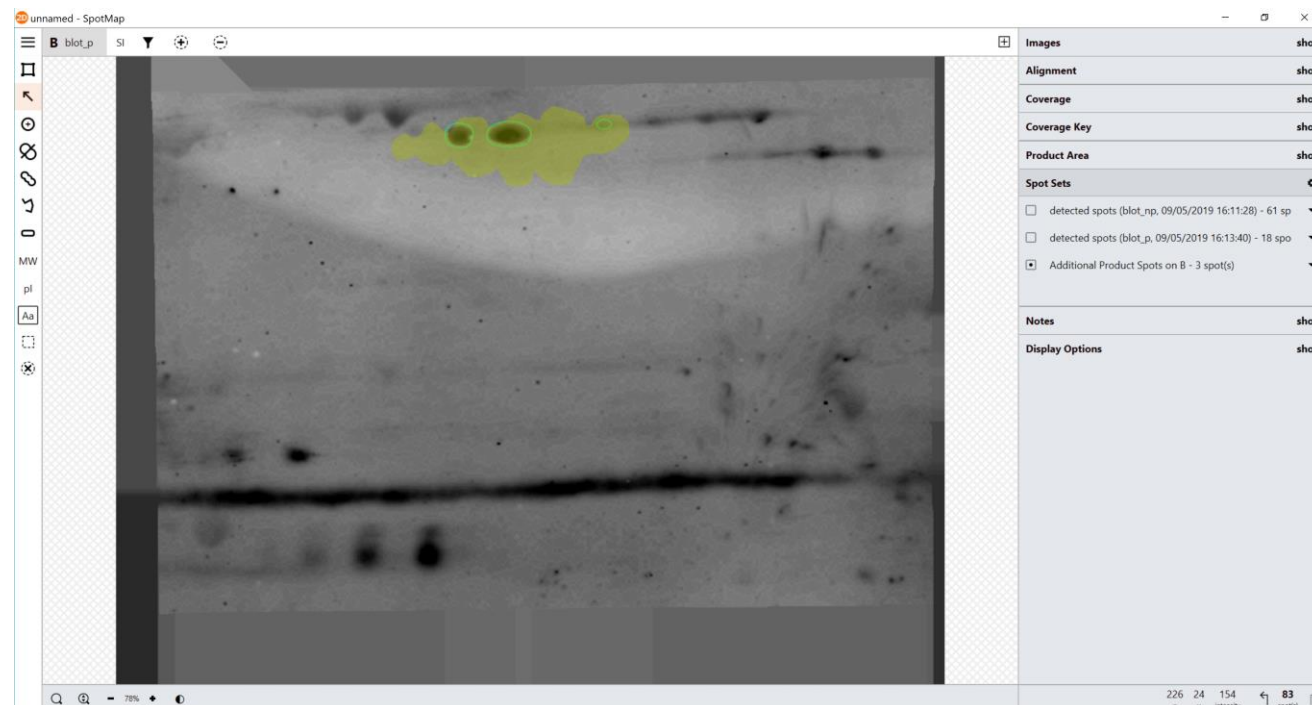


For more information, please email michael.hutchins@totallab.com



Set Coverage Target
to Blot image
(without product).

Look for Additional
spots on Blot image
(with product) and
click Create Set.

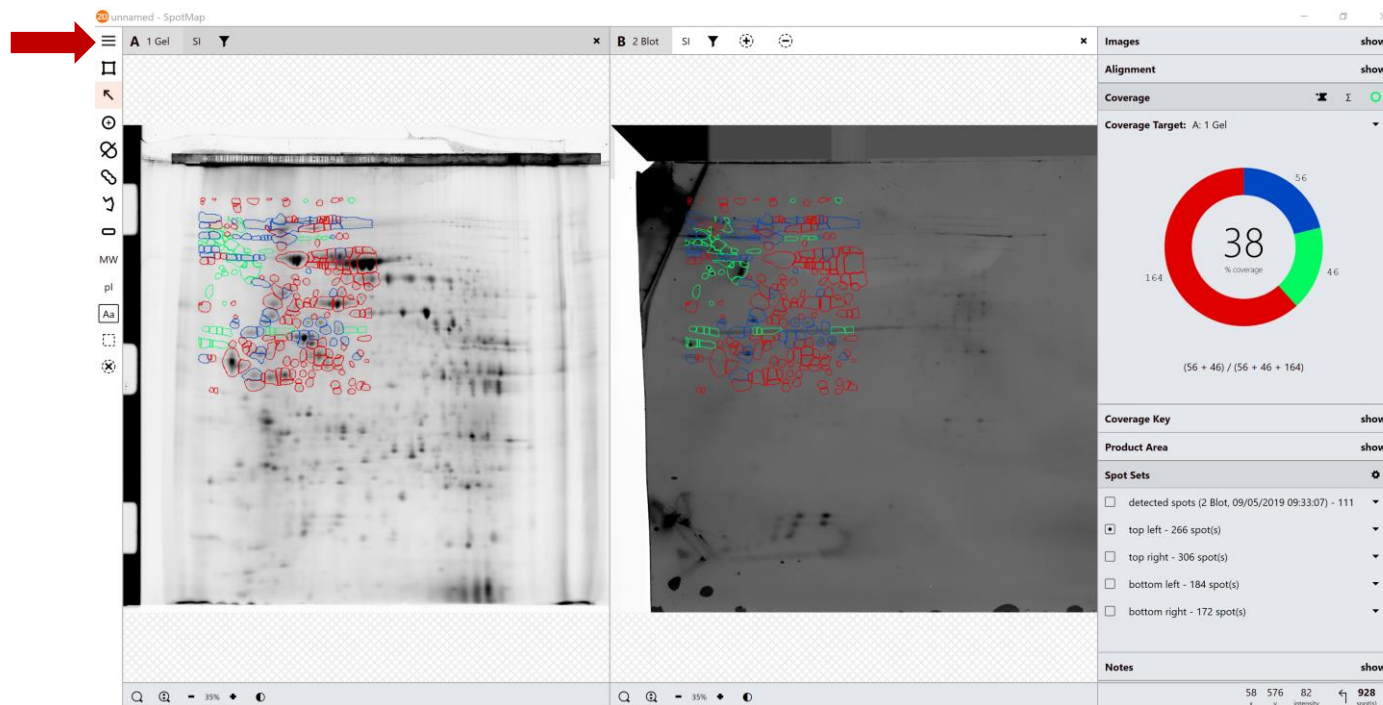


Any new spots on the Product Blot (compared with the non-product Blot), within the product area, are generated as a new Spot Set.

Saving projects & Data Access

For more information, please email michael.hutchins@totallab.com

Click this button (3 lines)
to access File menu



For more information, please email michael.hutchins@totallab.com

File Menu:

| | |
|--------------------------------------|---|
| • New | Create new Project |
| • Open | Open existing saved Project |
| • Save | Save current project (overwrite smp file) |
| • Save As | Save current project (new smp file) |
| • Data Access | Open Data Access panel |
| • Save Audit Trial As... | Save Audit Trail to pdf |
| • Reveal Project in Windows Explorer | Open location of Project (smp) file |
| • Welcome to SpotMap | Open first window |
| • Close | Close software |

Data Access panel:

1 - Data Access

Working Image: B: 2 Blot

Reference Image: A: 1 Gel

Spot Set: [all spots]

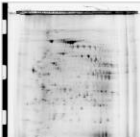
Total Number of Spots Absent in Working Image687

Total Number of Spots Absent in Both Images0

Measurements Table[CSV Table Data]

Audit Trail[CSV Table Data]


▼ Images



☐ Detected Spots
 ☒ Edited Spots

☒ Gel Spots
 ☒ Blot Spots
 ☒ Common Spots
 ☒ Absent Spots
 ☒ Annotations
 ☐ Detection ROI
 ☒ MW/pI Scale

Reference Image



☐ Detected Spots
 ☒ Edited Spots

☒ Gel Spots
 ☒ Blot Spots

Working Image

Create PDF Report

☐ Generate High DPI Images

The Data Access panel is where you can access your data.

All images can be customised and dragged to your desktop as a 300dpi bmp file or dragged to most Microsoft applications (including Word and Powerpoint).

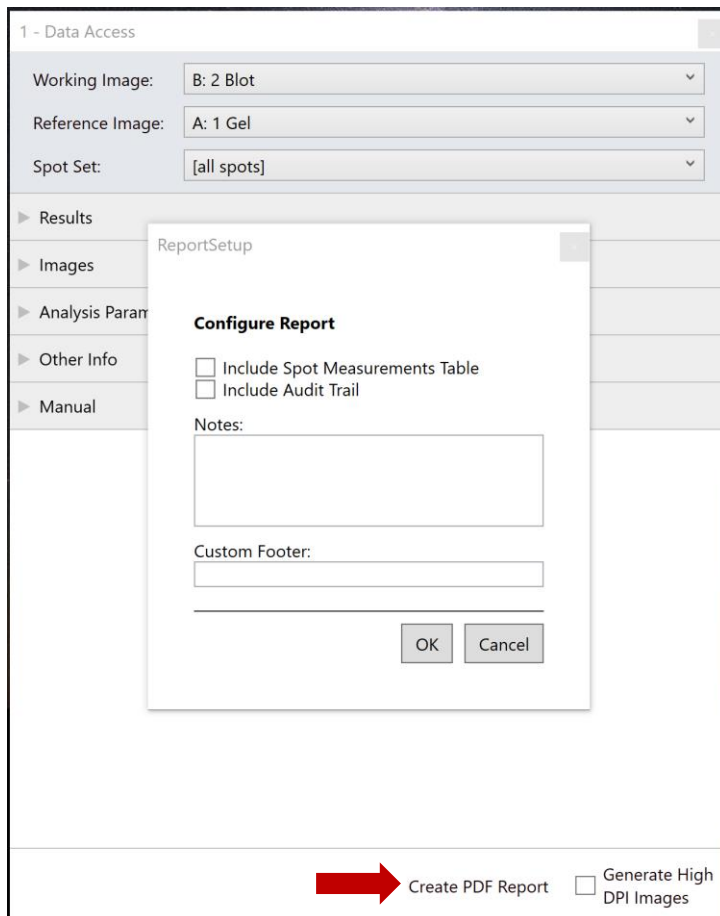
Text information can be dragged to most Microsoft applications – for example a pre-existing Word template for an HCP coverage report.

CSV files can be dragged to the desktop and opened immediately.

Most images and other data can be saved throughout the analysis simply by clicking and saving to Data Access. They will appear in the Manual tab.

600dpi images can be saved by ticking the Generate High DPI Images box.

PDF Report:



1 - Data Access

Working Image: B: 2 Blot

Reference Image: A: 1 Gel

Spot Set: [all spots]

Results

Images

Analysis Param

Other Info

Manual

ReportSetup

Configure Report

☐ Include Spot Measurements Table

☐ Include Audit Trail

Notes:

Custom Footer:

OK Cancel

Create PDF Report ☐ Generate High DPI Images

By clicking the Create PDF Report button, you will be able to generate a PDF report from the project, including:

- The measurement table for all Spots in the project
- An Audit Trail of all user inputs
- Custom notes field for the project (This can also be accessed in the Notes Tab within the software analysis window as well)
- Custom footer (Appears on every page – for LIMS users requiring an experiment number on every page)
- Any manually saved images throughout the experiment
- Any image notes (accessible through the down arrow next to the image name in the Image tab within the software analysis window)

The PDF Report name is the same as that of the saved project. If the project has not been saved, it will be unnamed.