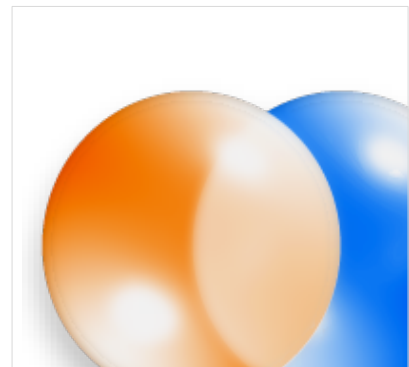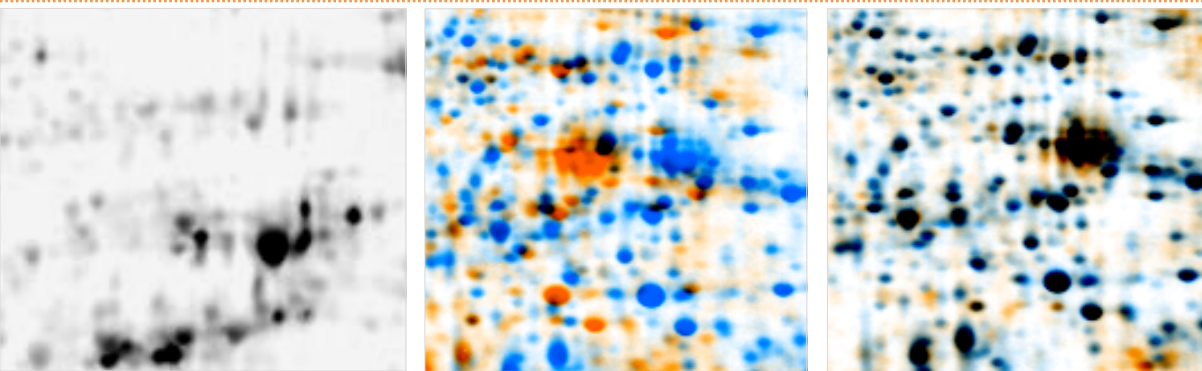# Getting started

English

2D Western Blots

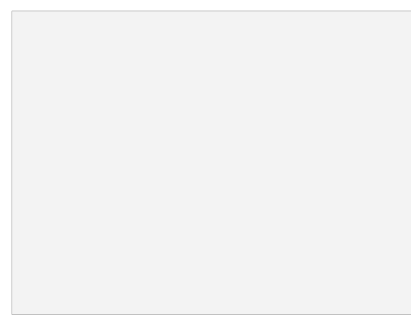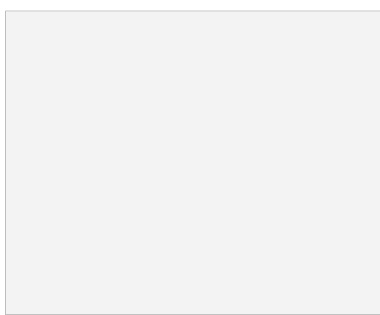# Delta2D

ANALYZING 2D GELS
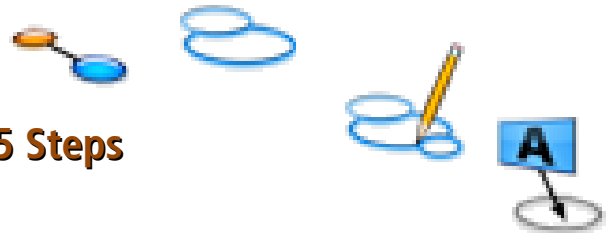AS EASY AS POINT AND CLICK

DECODON

DECODON

Analyzing 2D Western Blots in just 5 Steps

1 Setup Project

Setup a Western Blot project, import and arrange the images in predefined groups.

2 Warp Images

Choose the 2D Western Blot Warping Strategy.

**3 Detect and Quantify Spots**

Create a Fused image containing Total Protein (gel) and Western Blot (signal on membrane). Detect spots and transfer the consensus spot pattern to both.

4 Analyze Total Protein and Blot

Find interesting spots by setting filters, or select manually.

5 Present Results

Use the Western Blot report to show coverages, reproducibility and many more features.

Getting started
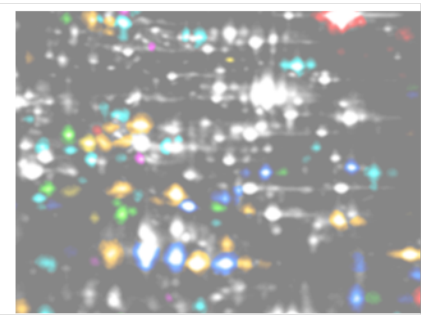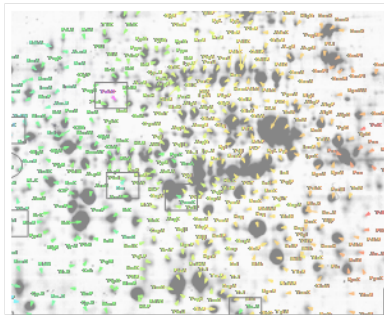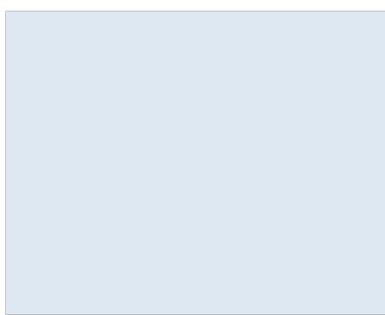
What's so special about 2D Western Blot projects?

Due to the quite different looks of Western Blots and gel images, it is not so easy to align these two image types for differential analysis. With a little workaround, this still becomes possible: by using support images, which are not analyzed themselves, but support a better alignment of the actual images. We use the outer shape of the membranes to align those images, whose signal pattern is not comparable. Therefore, to align one Western Blot to the gel image it is derived from, we need a set of 5 images:

1. **Total Protein Gel**
   actual gel image of the total proteins
2. **Total Protein Membrane**
   image of the membrane after blotting with protein pattern, easy alignable to the gel image
3. **Total Protein Membrane Contour**
   image of this membrane after blotting, focuses on the contour, alignment is identical to 2.
4. **Western Blot Contour**
   image of the membrane after the 2nd antibody incubation for the contour, can be aligned easily to the contour image 3.
5. **Western Blot 1** (up to 3 possible)
   image(s) of this membrane after the 2nd antibody incubation (antibody signal), alignment is identical to contour image 4.
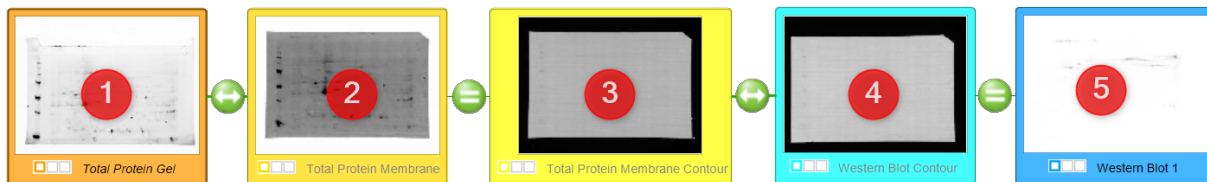
While image 5 is the the Western Blot and image 1 the actual gel image, we want to align it to, images 2 to 4 have the role of support images for a precise guidance from 5 to 1.

Delta2D's special project setup now considers the different functions of the images and treats every image according to its role in this setup. To identify the role of an image, Delta2D looks for the role names **Total Protein Gel**, **Total Protein Membrane**, **Total Protein Membrane Contour**, **Western Blot Contour**, and **Western Blot 1** (optionally also Western Blot 2 or Western Blot 3).

Depending on your project setup, this can be in two places:

1. If there is only one set of 5 images in the project, and these images are distributed according to their roles on 5 *groups* with exactly these *names*, this is distinct enough for Delta2D to recognize the roles.
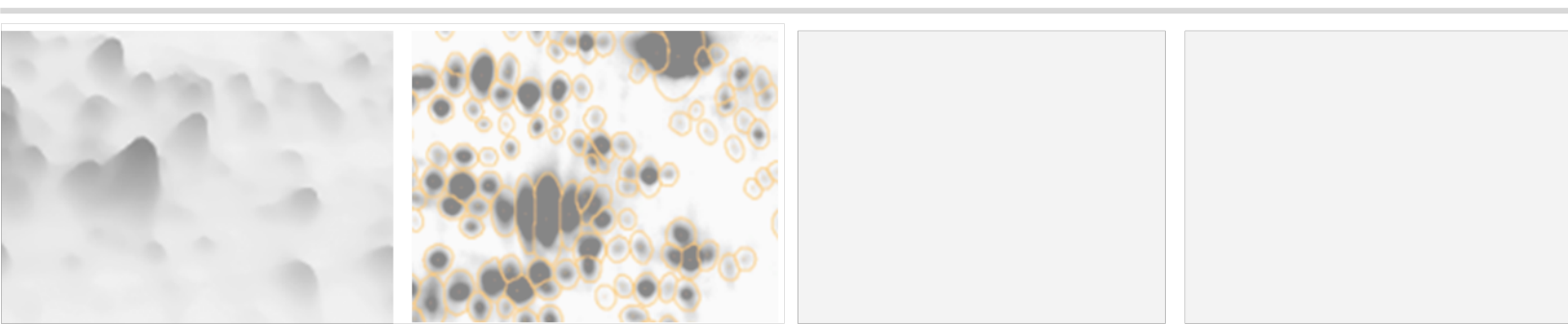
2. If you prefer a different grouping, e.g. all images in one group, or you want your groups to have different names, Delta2D uses the *sample* assigned to each image to identify its role. In this case it is crucial, that you *assign* each image to the *correct sample*.

On creating a project of the type "2D Western Blot", Delta2D always tries to do the assignments during the image import procedure automatically on the basis of the image names. Please check the proposals in the respective fields of the Import Wizard.

Multiple Sets in one Project

Of course you can have multiple sets of this kind in one project. In this case, Delta2D not only has to be aware of the role of each image, but also to which set it belongs. The set is determined by the image attribute "*Gel*". Hence it is important, that

- all images belonging to *one set* have to be assigned to the *same Gel*.

Advanced Techniques

If you want to include multiple (up to 3) Western Blots in one set, Delta2D needs additional information:

- The Western Blots 1to 3 have to be assigned to different channels.
- When using also multiple Western Blot Contour images (role 4), they have to be assigned to the same channel as their corresponding Western Blots:

    Western Blot Contour 1: Channel 1, Western Blot 1: Channel 1
    Western Blot Contour 2: Channel 2, Western Blot 2: Channel 2
    Western Blot Contour 3: Channel 3, Western Blot 3: Channel 3
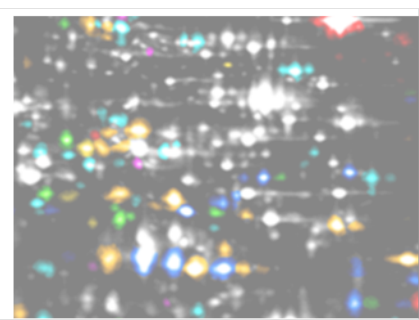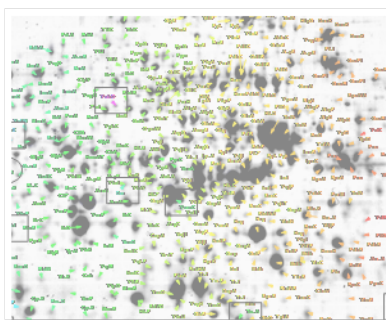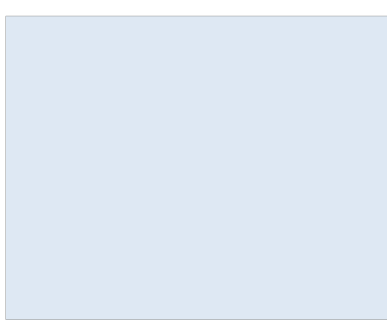
Setup Project

Create a new data pool

3. Click on the button **'Change Pool'** and *navigate* to a folder where you want to store your new pool.
4. Right click and select **'Create Folder'**, then click on the new folder to *rename* it. Type in a name for your new pool.
5. Click on OK and confirm the following dialog with Yes.
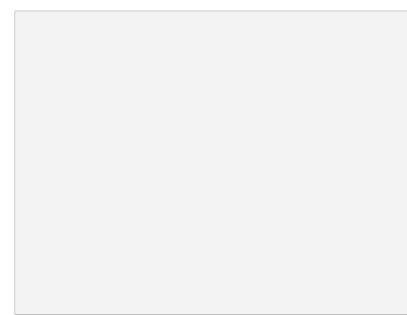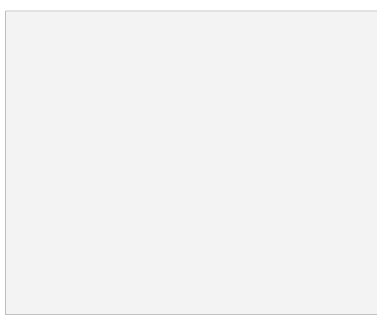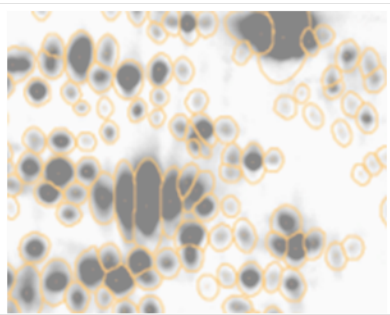
Create and open a new project

The **'Projects'** dialog appears again showing an empty list of available projects.

1. Click on the New button to create a new project.
2. Provide a name for your new project, author and choose "2D Western Blot" as type. Confirm with OK.
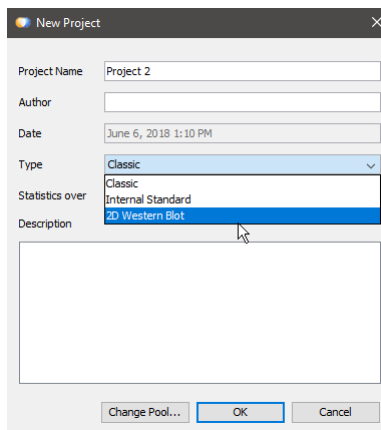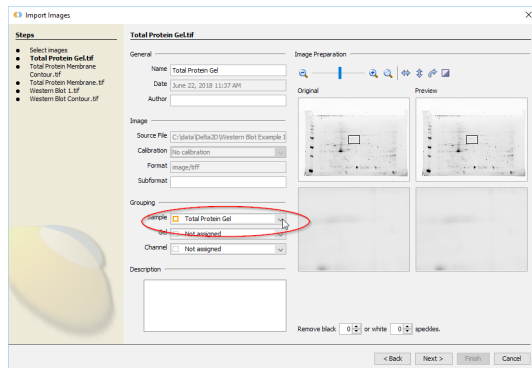

Delta2D's Projects dialog.

3. Select the new project from the list and press Open.

Import the images to the respective groups


Step 3: Details of each image to import

1. In step "Setup Project" of the Workflow click on the 'Import...' link.
2. Switch to the respective location and select all images you want to import. Click on [Next] to proceed to the first image.
3. Eventually edit the name, and make sure the image is assigned to its appropriate sample:
   - Total Protein Gel,
   - Total Protein Membrane,
   - Total Protein Membrane Contour,
   - Western Blot Contour, or
   - Western Blot 1/2/3.

4. If necessary, apply basic manipulations such as rotating, inverting or de-speckling to the image.
5. Click on [Next] and repeat steps 3 and 4 for every image.
6. Click on [Finish] to save assignments and close the dialog. The images will be automatic sorted into the groups, which are automatically created on choosing this project type.

NOTE:
The correct assignment of the images is crucial for this special project setup. If done during the import process, all images are sorted automatically into the respective groups. If you missed this step, you can resume it by clicking on "*Image Attributes*" in the workflow. Switch to the "*Sample*" tab in the "*Image Attributes*" dialogue, and drag the respective image to the desired sample and drop it there.
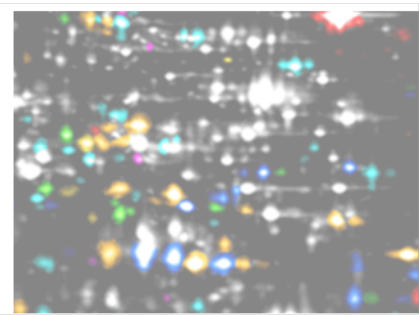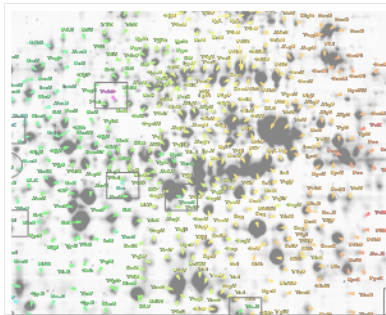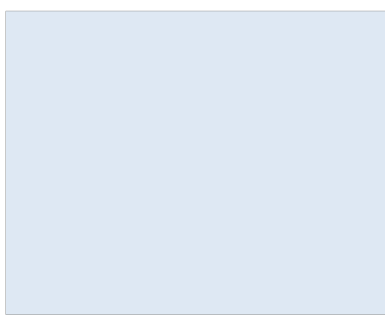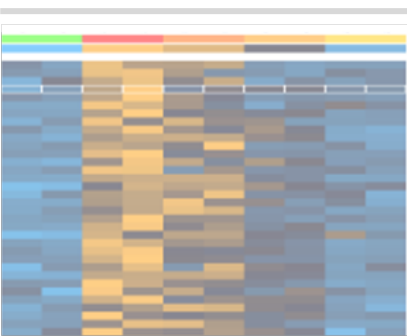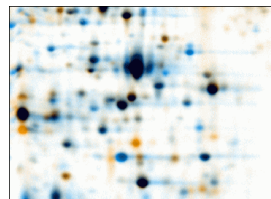
② Warp Images
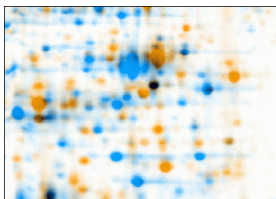
Two gel images, overlaid into a dual channel image, not warped.

Two gel images, overlaid into a dual channel image, after warping. Differences in expression levels are clearly visible.

Define a warping strategy

1. Switch to the second step of the **'Workflow'** called **'Warp Images'**.
2. Click on the 'Warp Strategy' link to open the **'Warping Strategy Manager'**.
3. Select the **'2D Western Blot Warping Strategy'** and confirm the default settings with OK .
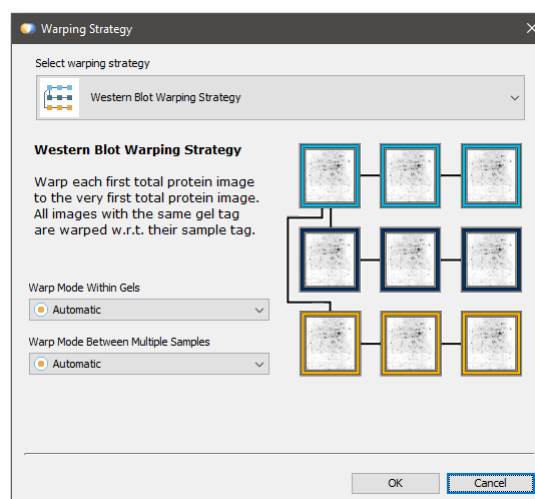
Delta2D's Warping Strategy Manager.

4. Open the **'Warping Setup'** window by clicking on the link 'Setup direct warpings' in the Workflow. It should look similar to the following image:
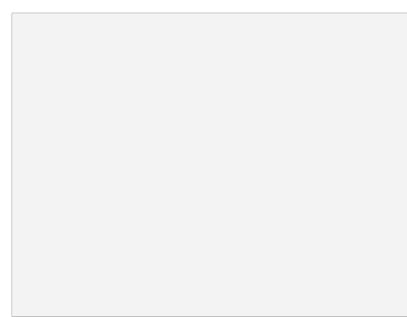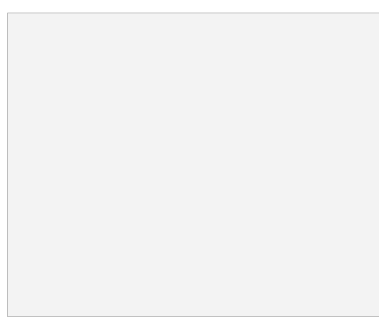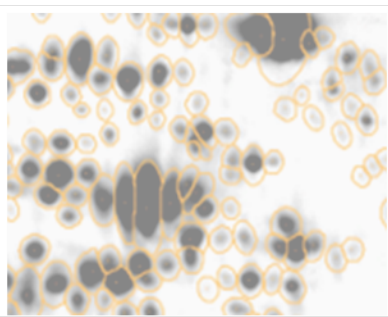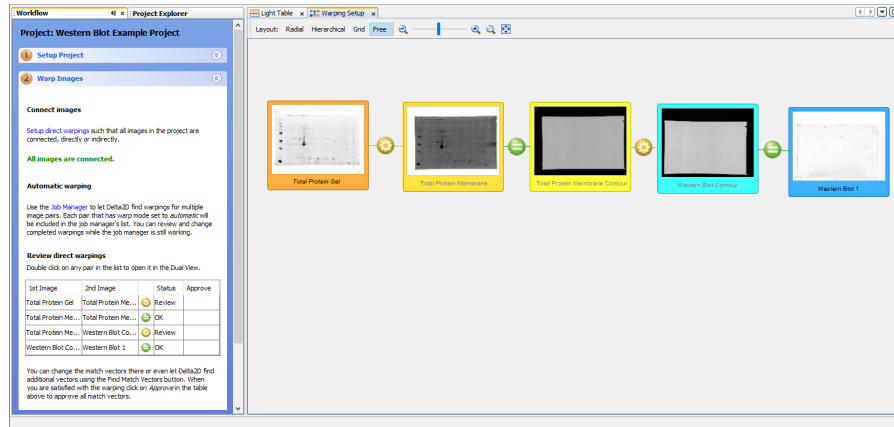
Warping Setup window after applying the **2D Western Blot Warping Strategy**.

Use Delta2D's Automatic Warping

1. If the **'Job Manager'** is not visible click on the 'Job Manager' link.
2. Just click the **'Start'** button ▶ in the **'Job Manager'**. It will monitor and perform all automatic warping jobs.

Review Warpings

Review direct warpings (Step 2 of the Workflow).

1. Double-click on a row in the table below **'Review Direct Warpings'** where the symbol looks like 🔄.
2. A **'Dual View'** window opens and shows the overlay of the respective two images.
3. Please also check if the image background has been hidden by using the **'Show/Hide Background'** button (▪) in the tool bar.

4. Review the automatic warping between Total Protein Gel and Membrane, as well as the 2 contour images. While Total Protein Gel and the Membrane should overlay the spot patterns best possible, the two contour images should simply match their shape.

Getting started
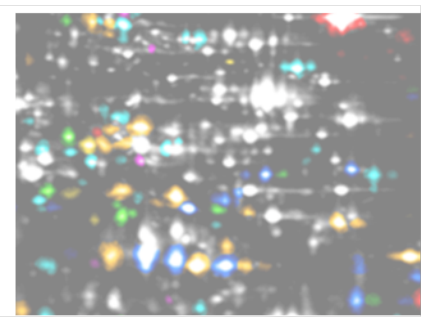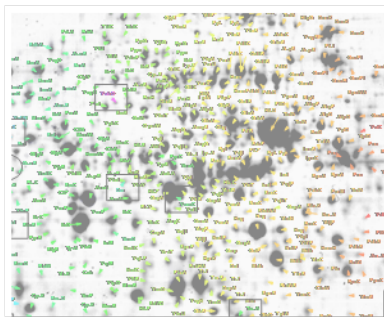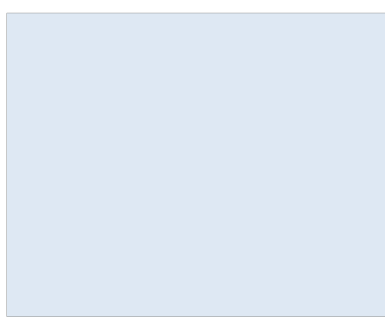
Note:

To work with match vectors, select the Match Vector Tool in the left vertical tool panel of the Dual View.
To ease the use you might want to switch on "Snap Match Vectors To Spots". This can be done at 'Tools' > 'Options' > 'Delta2D' > 'Match Vectors'. Temporarily switch it off with CTRL key.

If there are regions *where vectors seem to be incorrect*, you can set and change all match vectors manually:

- **Select a vector** – just left-click on the vector.
- **Select multiple vectors** – drag a rectangle with the mouse (keep the left mouse button pressed). All vectors in that rectangle will be selected.
- **Delete match vectors** – right click on a vector and select 'Delete' or 'Delete Selected'.
- **Set a new match vector** – first click on the spot in the orange, then on the corresponding spot on the blue image.
- **Change a match vector** – drag one end of the vector to change its position.

Note:
You can undo match vector operations by clicking the 'Undo' button .

Click on the Warp button to apply your new vectors. You can iteratively add, correct or delete match vectors until the result is satisfying.
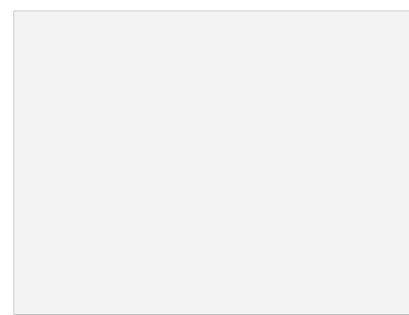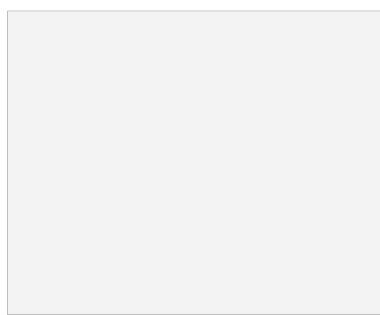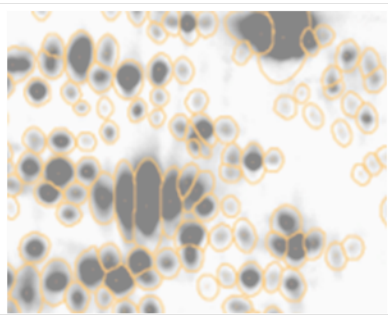
③ Detect and Quantify Spots

Generate a fusion image

1. Switch to the workflow step called **'Detect and Quantify Spots'** and click on the 'Fuse images' link to open the **'Image Fusion'** dialog.

2. Click on Fuse to use the default settings (Total Protein Gel and WB signal images) and let Delta2D create a new image.

Detect spots on the fusion image

Click on the link 'Detect Spots on Fused Image...' in the Workflow.

After detection is finished, the fused image will appear with detected spot boundaries.

Edit spots on the fusion

1. Open the new fusion image by clicking on 'Open Fused Image using Max ....'.
2. A **'Dual View'** window opens showing the single view of the fused image only.

Note:

Select the 'Spot Editing Tool' in the left vertical tool panel in the Dual View to edit spots.

We strongly recommend to edit spots ON THE FUSION IMAGE ONLY.
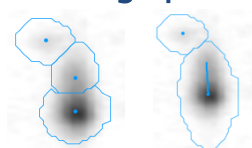
Adding a new Spot

Image region before / after adding a spot.
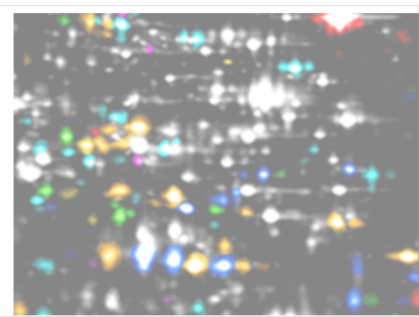(Just click on the center of an undetected spot to add it.)
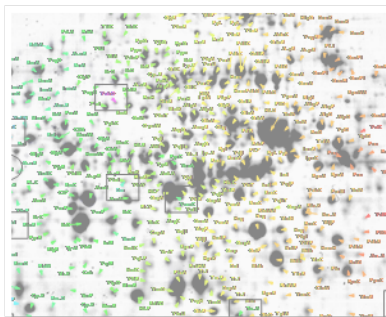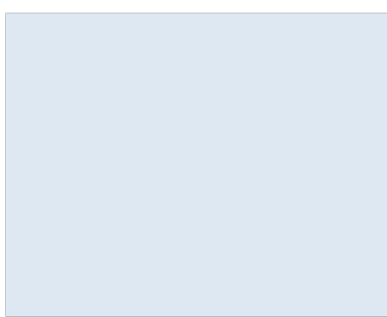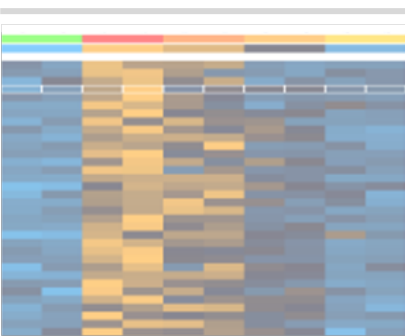
Splitting a Spot

Two spots, detected as one and split.
(Click once into the center of the spot that was not detected separately).

Joining Spots

Example for joined spots.
(Drag a line from one spot center to the other.)

Getting
started

Move Spot Markers

Spot markers can be moved by dragging with the left mouse button.

Delete Spot Markers

To delete a spot marker you can *right-click* on it.

Remove Spots

1. Activate the **'Spot Selection Tool'** and select one spot or a group of spots.
2. Right-click and select **'Cancel Spot' / 'Cancel Selected Spots'**.

To see all canceled spots with dotted line spot boundaries, select **'Spots' > 'Show Canceled Spots'** from the Dual View's menu.

NOTE:
You can easily assess a spot with the help of the 3D view (Dual View menu → Rollups --> 3D spots). Switch to the S*pot Selection Tool* and click on the spot or in the region you want to inspect.
In the overlay of Fused and Western Blot image you can directly add spots on the Fused image.

Transfer Spots

1. In the Workflow step **'Detect and Quantify Spots'** click on the link 'Transfer Spots from Fused Image...'.
2. The **'Transfer Spots'** dialog opens. Confirm the default settings with OK.
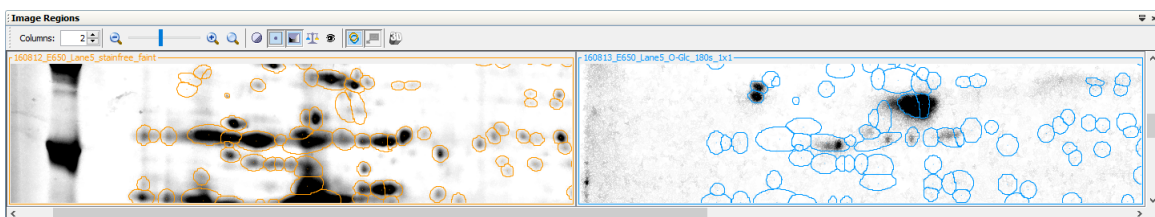
Spot boundaries after spot transfer. Choose the 'Gel Image Regions' link in the workflow to open this view. The essential spot pattern is the same on all gels, making 100% spot matching possible.
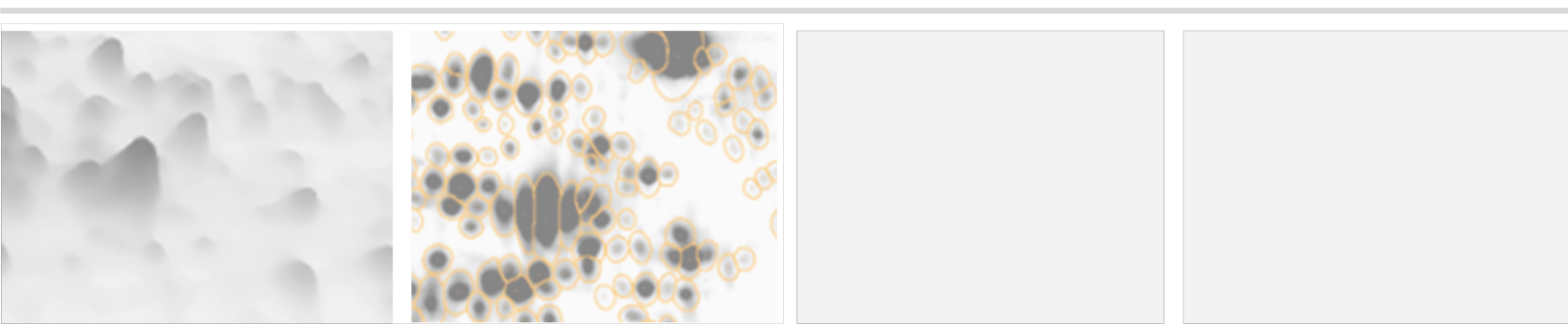
④ Analyze 2D Western Blot vs Total Protein image

Filtering for AB signal vs. Total protein

Note:
The Quantitation Table is synchronized with all other views of Delta2D: Selecting an expression profile in the table will select the spot e. g. in the Dual View.

To calculate the coverage we have 3 different kind of spots:

- only present (unmatched) on the total protein gel image
- only present (unmatched) on the WB image
- matched on both images.

Click in the Workflow step 4 on Quantitation Table to open this table. To show or hide the parameter, click on the Eye 👁 and choose the respective parameters by activating the checkbox below the name for all images and confirm this with OK. The Quantitation Table will be automatically refreshed.
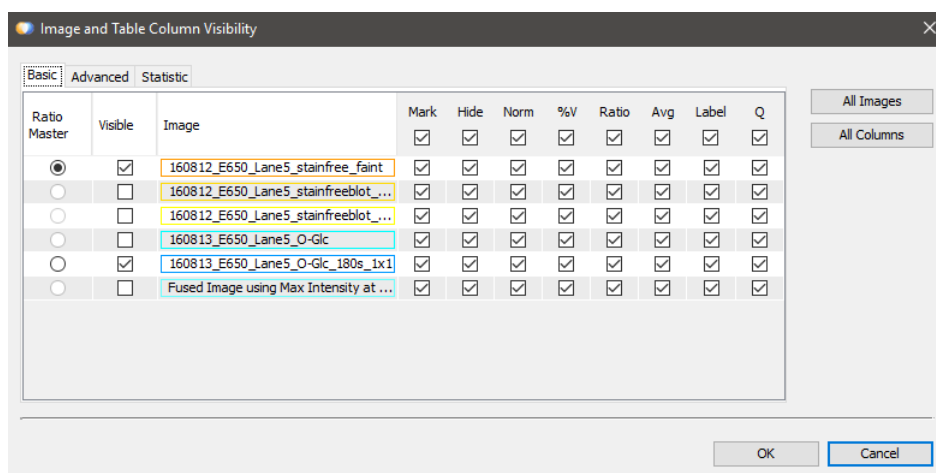
Control Visibility of Images and Spot Data

To define matched spots, the rest of the spots must be *cancelled or filtered*. Choose one of the parameter, sort the values by size, and use the filter (above the column) to enter border values.
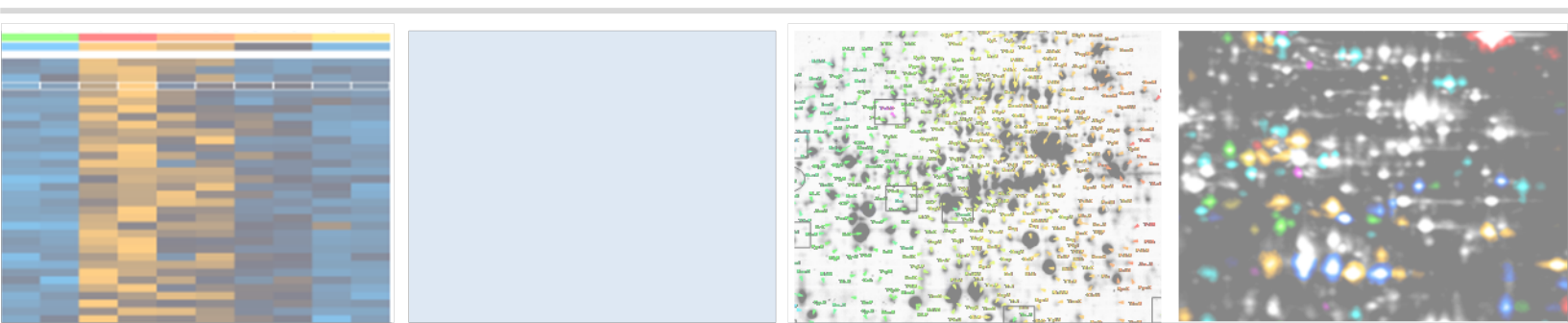
Available parameters are

- Volume **V** (sum of gray values within the spot boundary)
- normalized volume **%V** (ratio spot intensity to total intensity of the image)
- area **A** (of the spot boundary in pixel)
- average **Avg** (Volume / area)

If you want to select the spots manually, you can use the
- 3D view (Dual view menu "Rollup" → 3D view   and
- Select Spots in the Dual View

To cancel spots (e.g. in the Western Blot image) select them e.g. in the Dual View, open a Quantitation Table for this image (in the Light Table right mouse click on Western Blot image and choose "Open Quantitation Table") and click in the menu "Cancel" → Cancel Selected Spots.

NOTE:
If you want to keep few spots and cancel the rest, you can use the inverse procedure: select the wanted spots in the *Dual View*, open the *Quantitation Table* for this image, and choose from the table's menu successively: *Edit → Invert selection*; *Cancel → Cancel selected*.

To filter spots among a certain parameter:
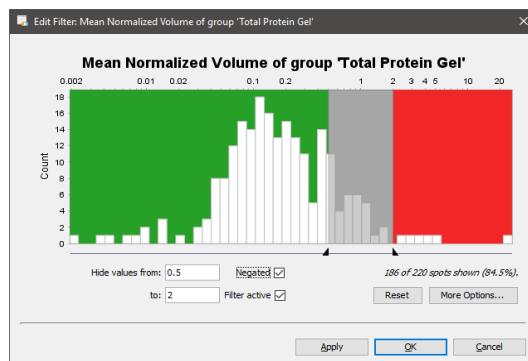
Filter Dialog for normalized volume %V

1. Click on the top part of the column header labeled **'Filter'**.
2. Insert the upper and lower filter borders **'Show values from'**.
3. The **'Filter active'** will be checked automatically, additionally check the box **'Negated'**. The latter will change the filter dialog's text to **'Hide values from'**. Confirm with OK.
4. Open the **'Dual View'** with the two images Only spots meeting our criteria will still have

spot boundaries.
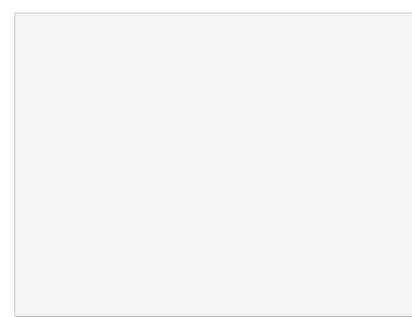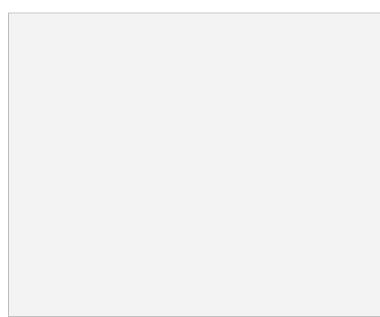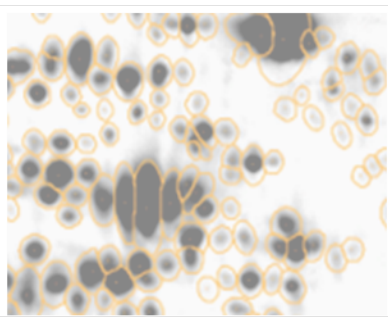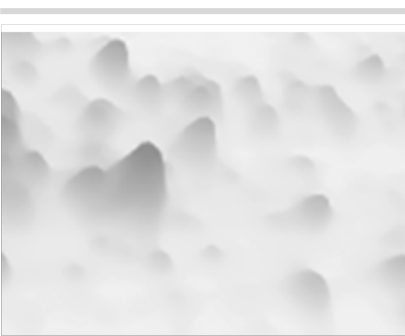
5 Present Results

Create special 2D Western Blot Reports

In step 5 "Present results", under *Reports* click on 🔲 *2D Western Blot.*

You can choose between:

- Using filters: The different paramters, lower and upper values set in Delta2D are shown,
- Using cancelled spots also defined in Delta2D's, or
- Using predefined parameters directly in the report (Delta2D settings have no impact)
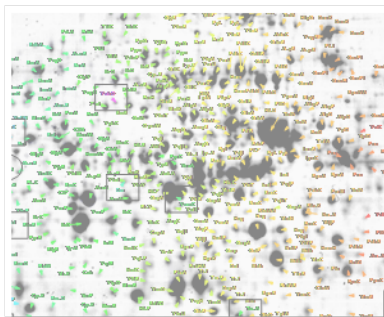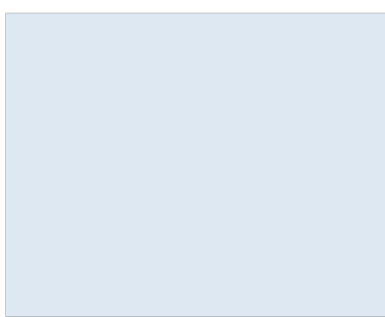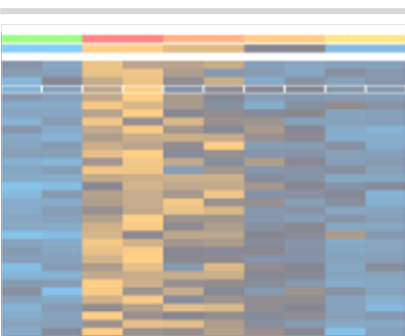

Enter a remark, check the filter settings (if applicable) and confirm your entry with [Submit].

Save and print this report

To save and print the report please click on [Save this report] on the top right corner of the report. After choosing a location and successful saving the button change into "Print".

Getting started
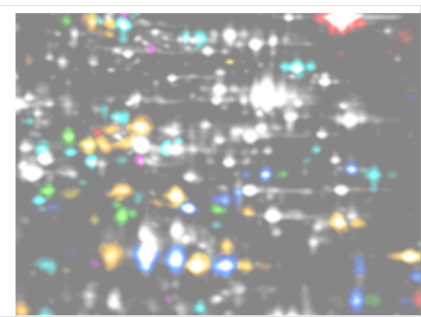
Where Can I Find Out More about Delta2D?

The easiest way to learn more is going to http://www.decodon.com or contacting us per Email to support@decodon.com. We will be happy to answer your questions or to show you more in a live web demo.

Note:
If you need more detailed information about Delta2D, please consult the manual of Delta2D. You can access it through the Delta2D menu 'Help' > 'Help …', or as a PDF file located in the installation directory. In Windows, there is a direct link to the manual in the Start menu. The manual is also available on our website for download and for online browsing!

Your DECODON Team

If you have any comment or suggestion regarding this **'Getting Started with Delta2D Guide'** or any other of our documents we welcome hearing from you.