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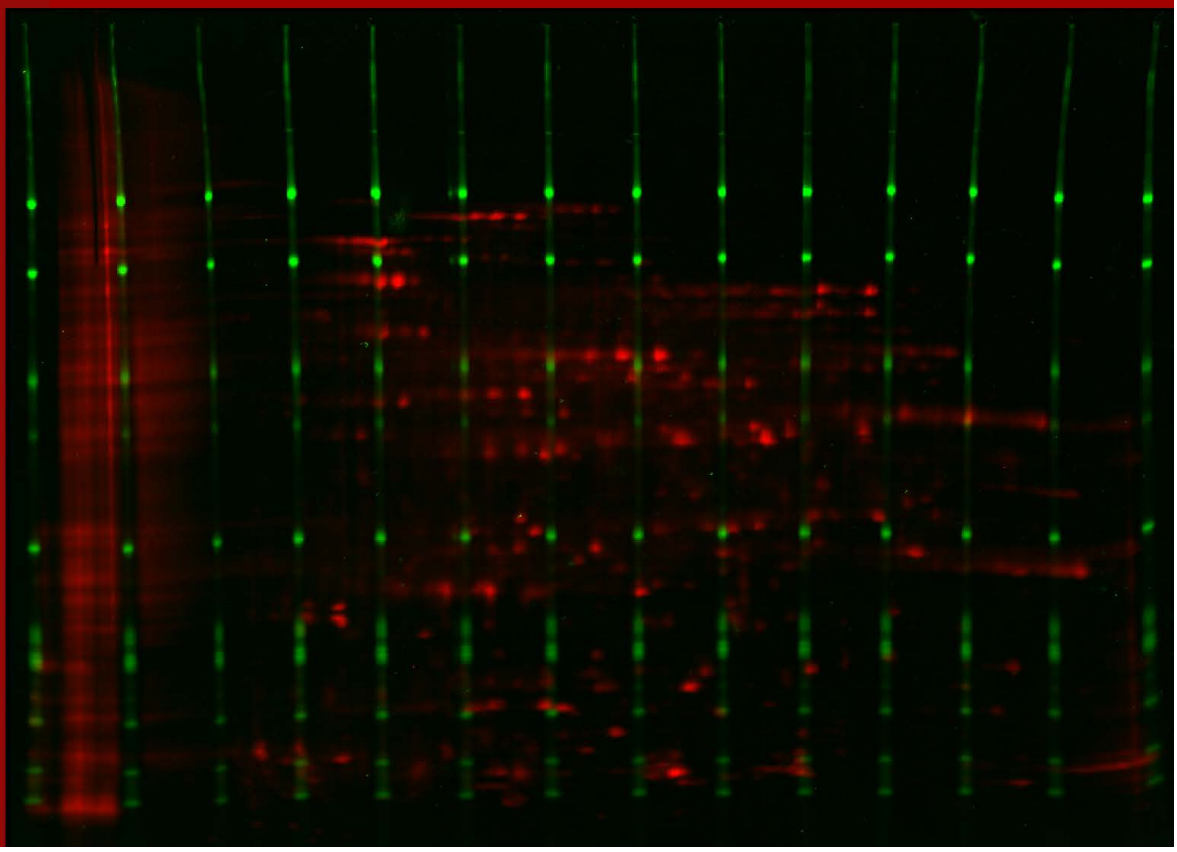

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Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Software Manual

Analysis of CoFGE experiments with Delta2D

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Abstract

Comparative fluorescence gel electrophoresis (CoFGE) was developed to improve coordinate assignment in singular experiments. The method uses an internal reference to both correct for gel-to-gel variation and allow semi-quantitation of gel spots in two-dimensional gel electrophoretic experiments (2D-PAGE). Commercial products have become available which enable users to easily perform these powerful experiments. Software capable of warping gel images is required for data analysis. A manual is provided for the use of Delta2D (DECODON) for this purpose.

Introduction

Comparative fluorescence gel electrophoresis (CoFGE) was developed for the reproducible assignment of protein spot coordinates after two-dimensional gel electrophoresis (2D-PAGE, [1-5]). By overlaying traditional 2D-PAGE with a 1D-PAGE reference channel it is possible to correct for gel-to-gel variation. The method was set up for singular samples and is thus complimentary to differential gel electrophoresis (DiGE), which uses replicates.

Precast gels were commercialized by SERVA Electrophoresis (Mercator-Gels, cat.# 43410.01). The software developed by the German company DECODON (Greifswald) allows data analysis.

This manual guides the novice through the procedure using an exemplary data set comprising four Mercator gels on which the *Escherichia coli* proteome and a set of standard proteins were separated. The data set thus consists of four proteome 2D-gel images and four corresponding reference grid images.

> Note: The analysis of four replicate gels represents an artificial situation used only for validation purposes here. CoFGE was developed for the comparison of gels, which were run at different points in time or in different laboratories. The link among them is the identical reference.

The goal of data analysis is primarily the export of corrected spot coordinates for the proteins of interest. In addition, semi-quantitative information about these proteins can be obtained by comparison to the internal standard with known concentration.

> Note: Download the Manual, the Getting Started Guide and demo images from the DECODON website (www.decodon.com) for general information.

There are two possible analysis approaches depending on the experimental scenario. Both are explained below.

- For projects, which continue over an indefinite period of time it is useful to generate a master gel as a fusion gel of at least three experiments performed right at the beginning of the project. This gel will then serve as a global reference.

- In case of smaller sets of gels within a temporary research project, one reference image can be selected as master reference to which all other reference gels are matched.

> Note: CoFGE relies on the use of the exact same lab procedure with respect to pI-strip and protein standard mixture. [1-5]

1. Generation of a Pool and a Project

After successfull installation, the program opens with the window shown in Figure 1. Demo projects in a demopool are listed.

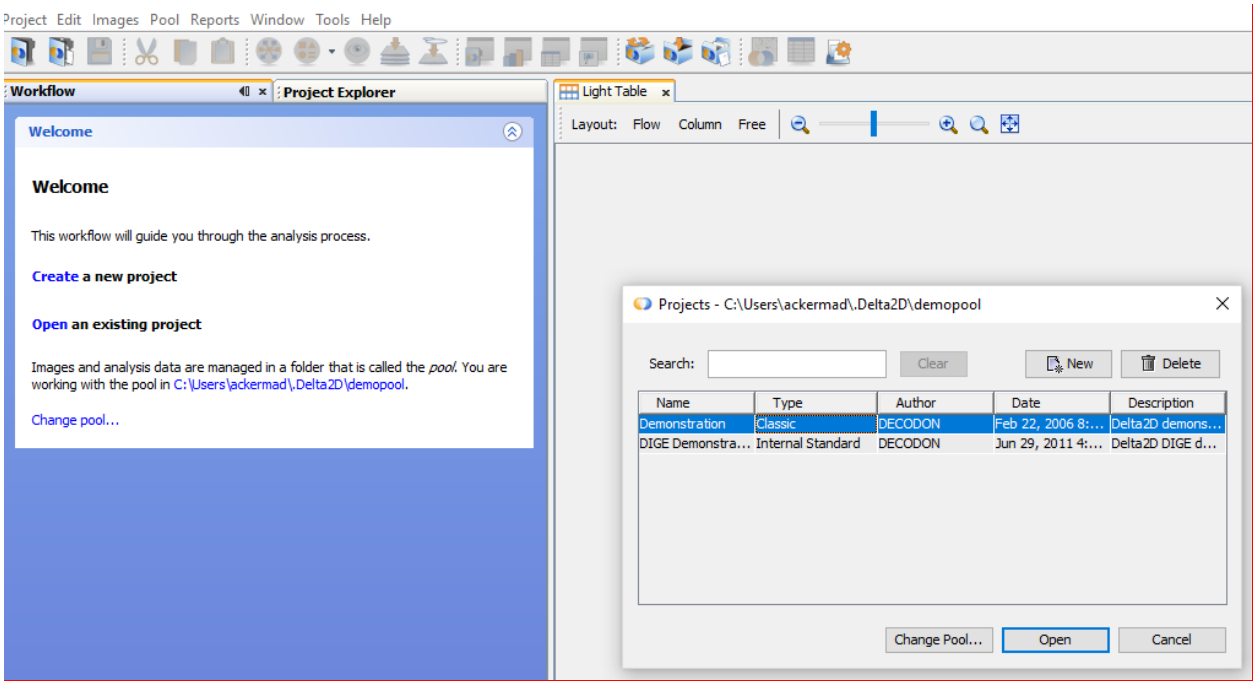


Figure 1: Start screen in Delta2D.

Delta2D works with “Pools” and “Projects”. A gel pool is a central depository for data such as projects, individual gel images, quantitative results, match vectors and labels. It is possible to create more than one project in a pool.

> Note: When the same gel image is imported into different projects within one pool, changes on the gel in one project may cause changes in the match maps of another project.

For the analysis of a new dataset, a new pool needs to be created. It is not advisable to use the existing demopool, because there is the possibility of errors or data loss.

The pool is best saved locally and not on a network to avoid difficulties due to network instability. The pool should be accessible to all who work with a specific project.

‘Change pool‘ (Figure 1) opens a new window (Figure 2), where name and storage location of the new pool are chosen. This pool does not have the necessary pool structure, yet. After confirmation with the ‘OK‘, the window shown in Figure 3 asks for confirmation of the data structure in this data folder.

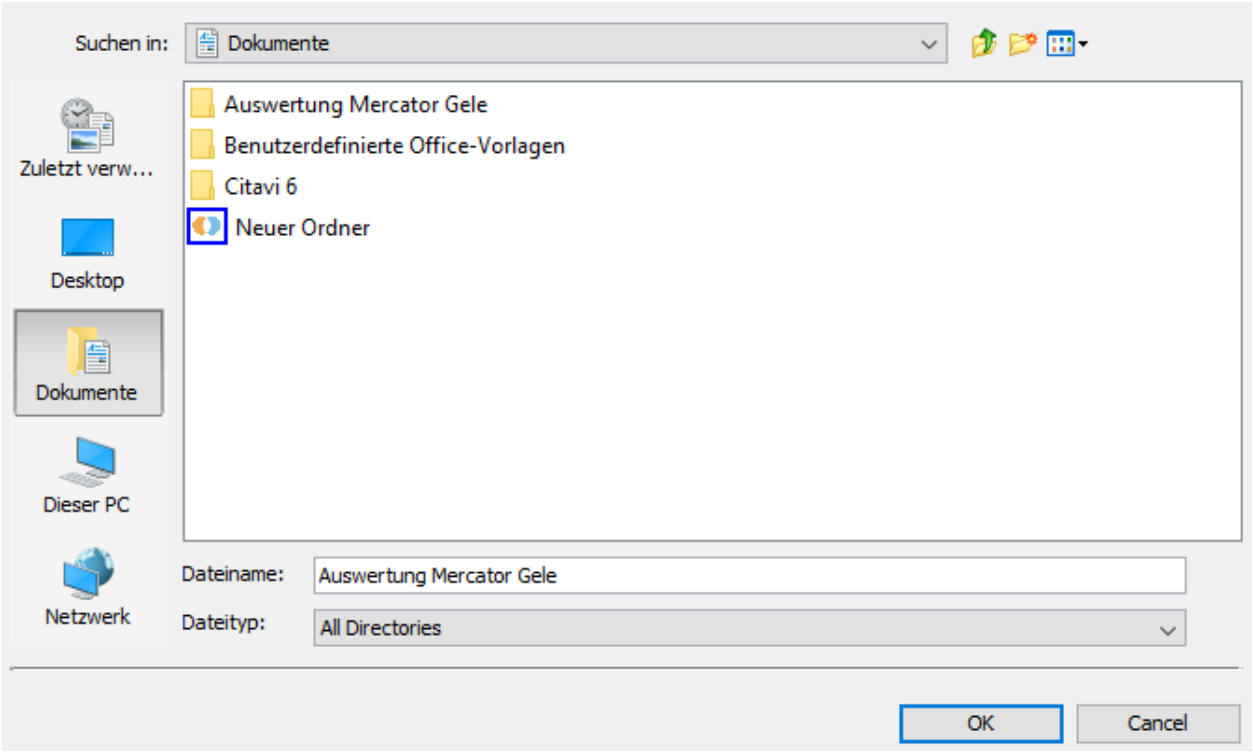


Figure 2: Creation of the new pool ‘Auswertung Mercator Gele’.

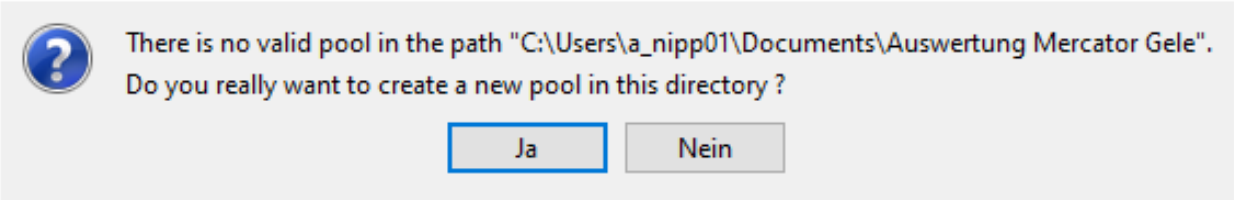


Figure 3: Confirmation of creating the pool data structure.

Figure 4 shows the folders, which are automatically generated, when a new pool is created and confirmed. Note that the folder symbol changes from to . After creating a pool, a new project in this pool needs to be generated (Figure 5). By clicking New the window shown in Figure 6 appears in which project properties can be defined. It is possible to change these properties later.

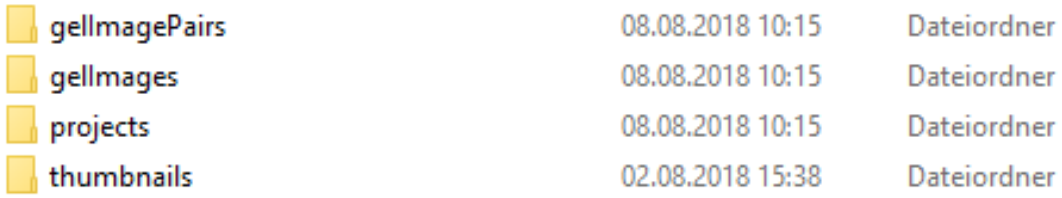


Figure 4: Pool data structure.

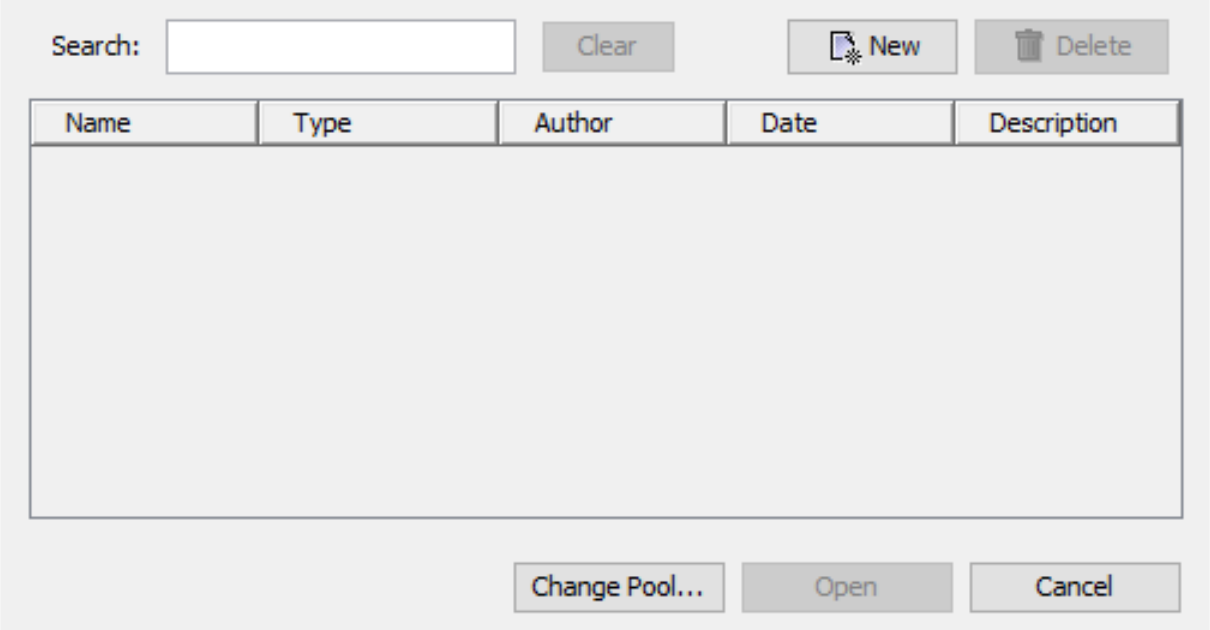


Figure 5: Project overview.

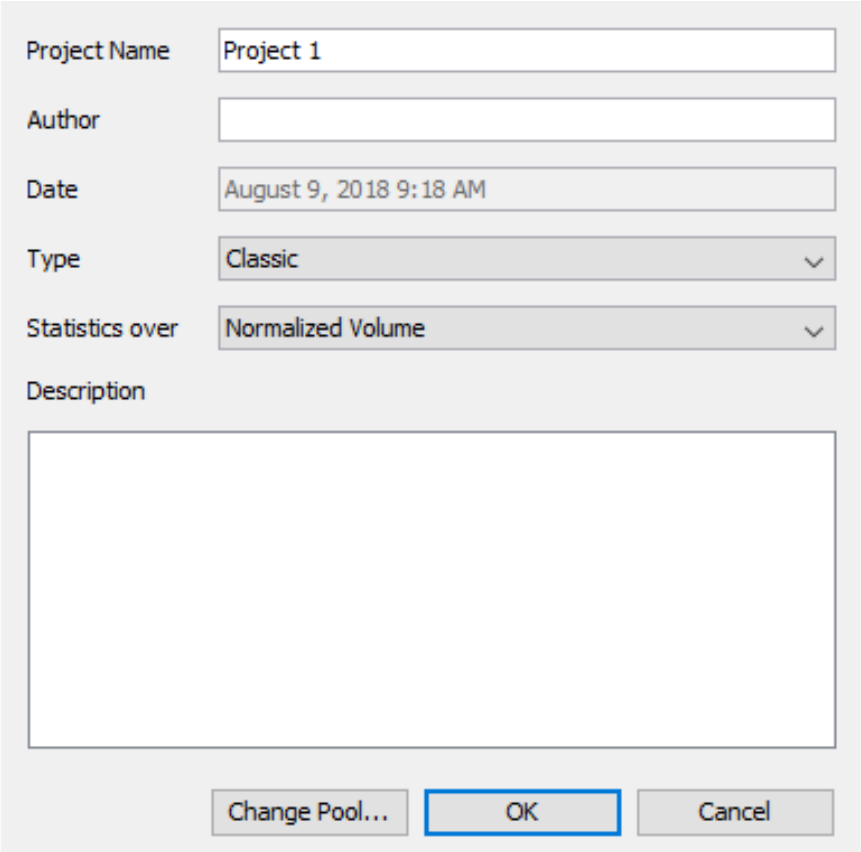


Figure 6: Project properties.

The project is shown in the pool overview (Figure 7) and it can be opened by pressing ‘Open’. A new view (Figure 8) is displayed.

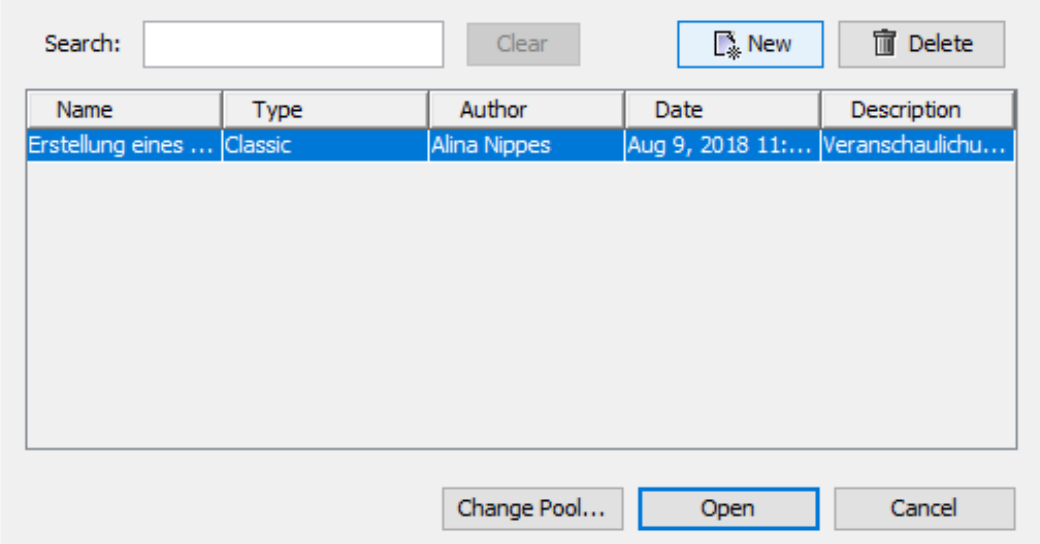


Figure 7: Project in pool overview.

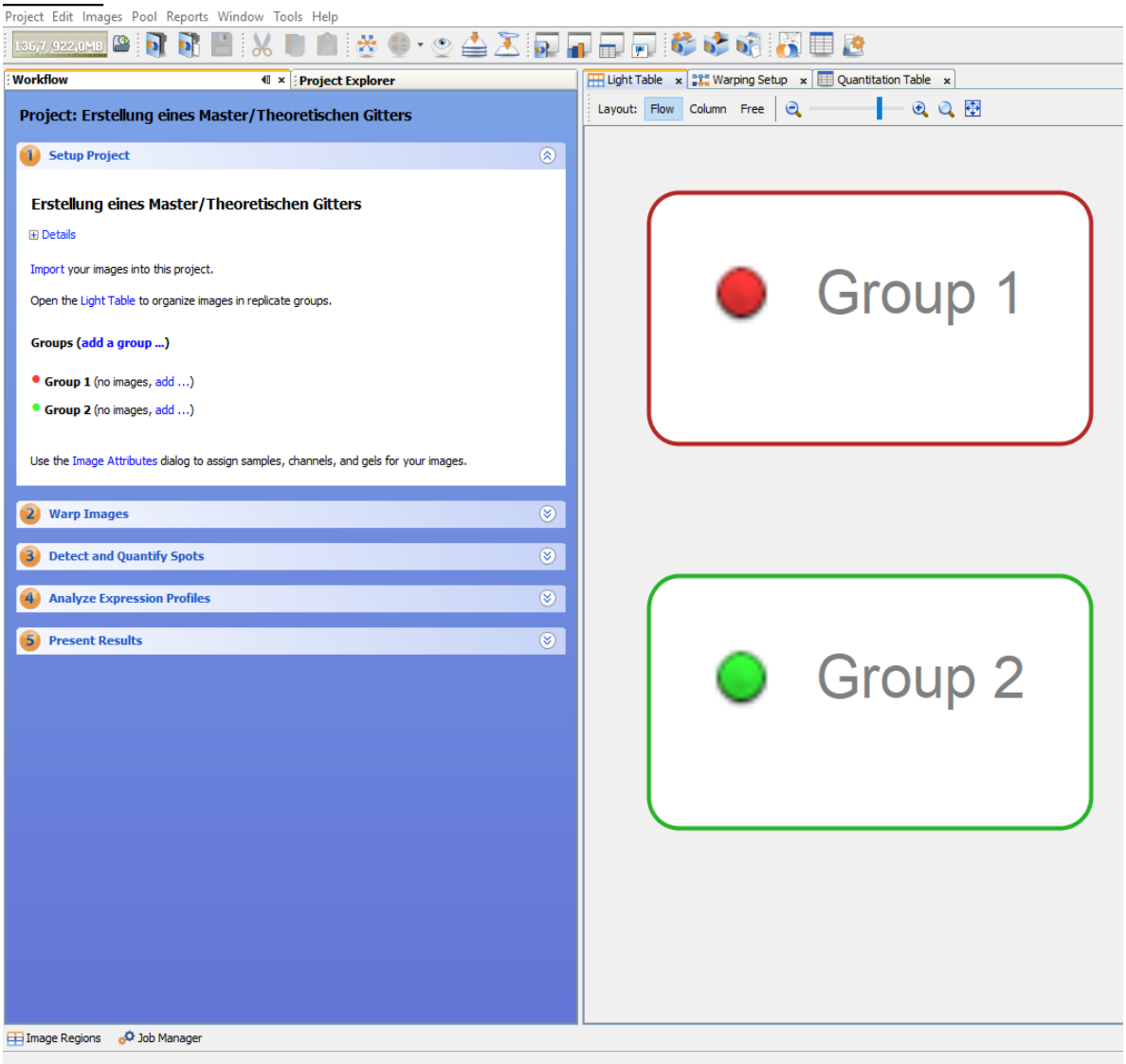


Figure 8: 'Workflow' and 'Light Table'.

2. Import Process und Image Activation

2.1 Importing Gel Images

In the project page (Figure 8), on the left hand side is the ‘Workflow’, on the right the ‘Light Table’, into which the gel images are imported and where they are handled.

In order to import images, they have to be added either in ‘Workflow’ under the subitem ‘1 Setup Project’ at ‘Import your images to this project’ or in ‘Light Table’ by holding the right mouse button.

Group assignment is not yet important; it can be changed later. An image can be moved from one group to another by pressing the left mouse button.

At this point, only gel images of the reference grids are imported. Later in the process, a new project will be created for the sample images.

Figure 9 shows the first import window. It is possible to select several images. In the next step (Figure 10), the function ‘Remove speckles‘ should be used to remove colour spikes which are only a few pixels in size. On white gels black speckles are removed and on dark gels the white ones. The value to use for fluorescent dyes is 2 to 5.

> Note: When a gel image is imported into a different pool, it is necessary to use the same despeckling value. Otherwise Delta2D will read it as a new image and the activation will not be free of charge. Thus take note of the despeckling value.

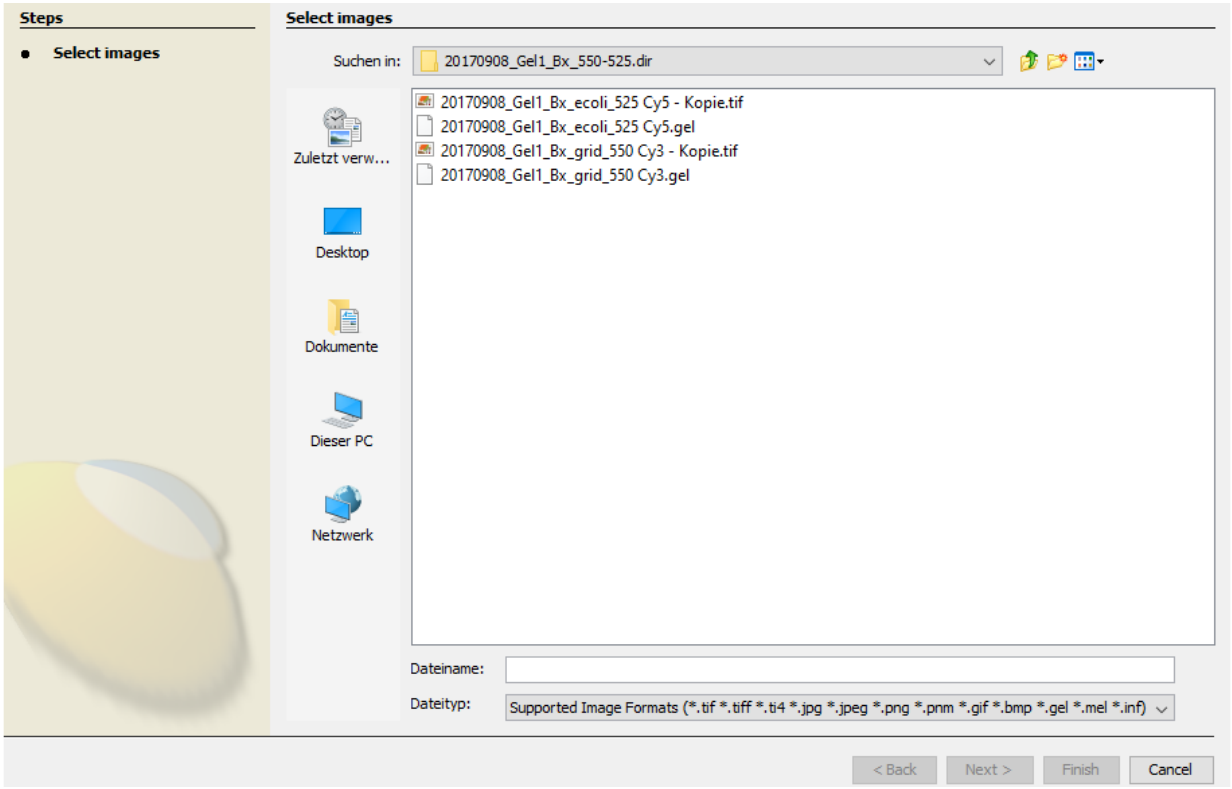


Figure 9: Import gel images, 1st window.

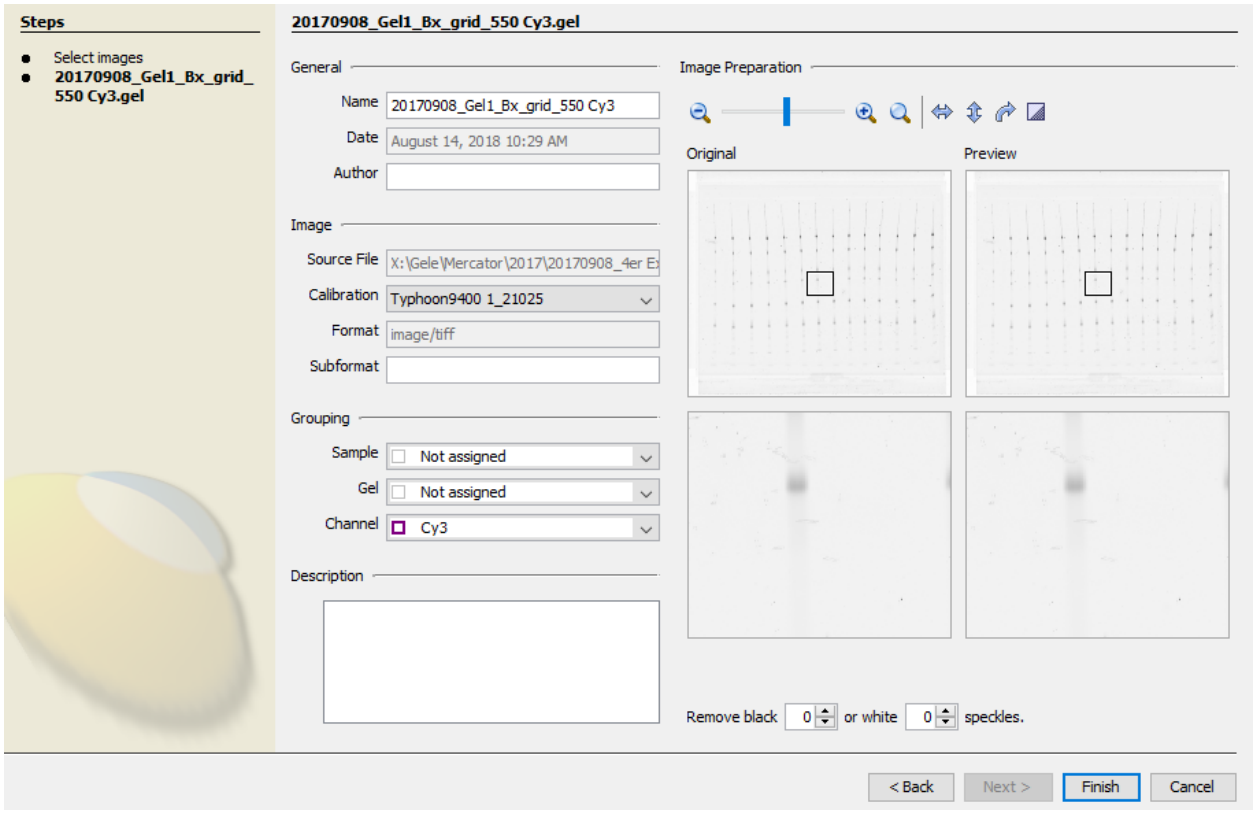
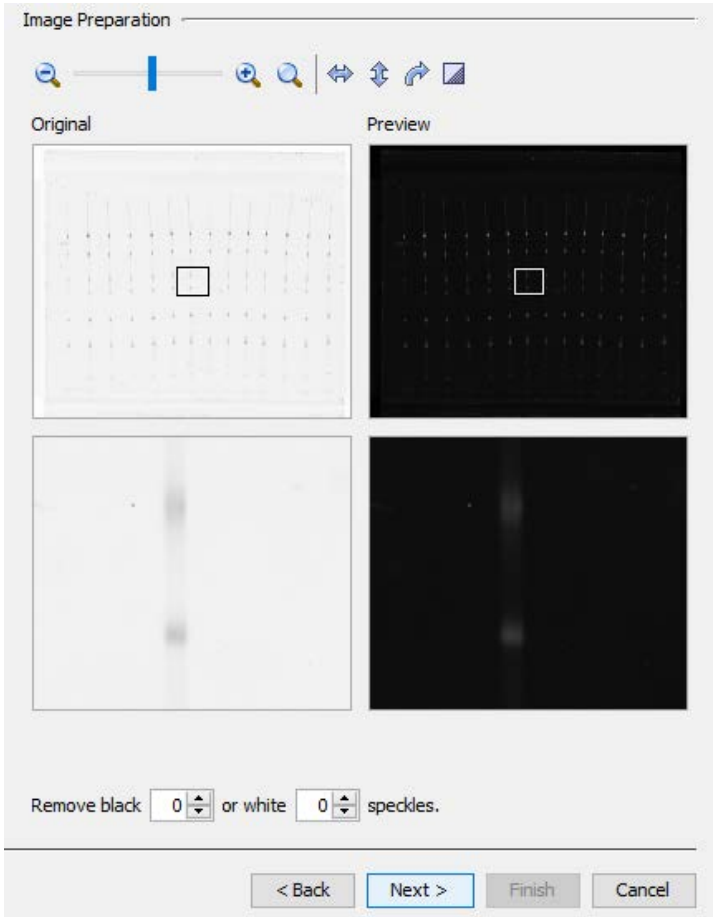


Figure 10: Import gel images, 2nd window.

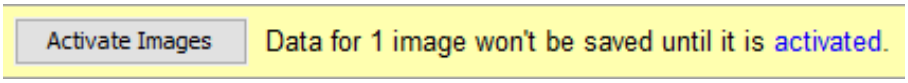


If gels appear black in preview (Figure 11) they have to be inverted using  .

Figure 11: Gel, black in preview.

If gel images have been imported into a project by mistake, it is possible to delete them in the 'Light Table' by right clicking the gel image and choosing 'Delete'.

After importing the gel images it is necessary to activate them



by entering username and password (Figure 12). Non-activated gel images are labeled with a padlock in the upper left of the gel image in the 'Light Table'.

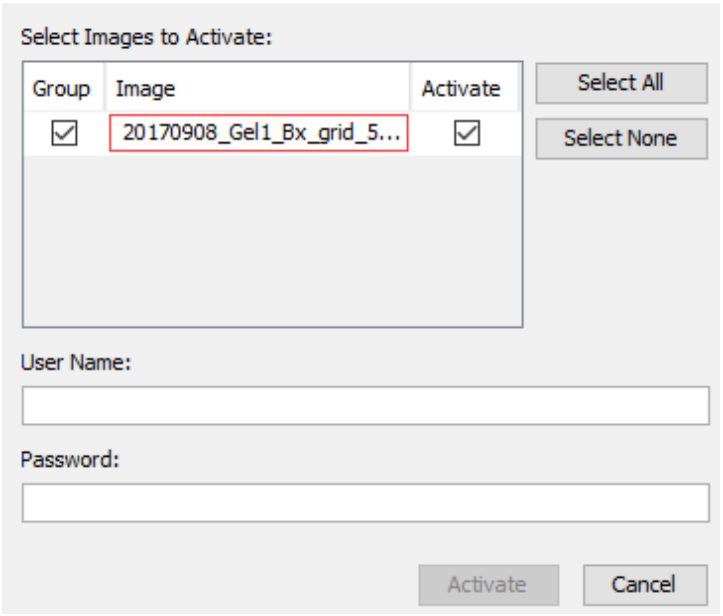


Figure 12: Gel image activation.

In this example workflow, there are four gels. Two more groups must thus be added to the 'Light Table' to handle them all (Figure 13). Groups are renamed in 'Name' to gel 1-4. It is also possible to rename by right-clickling on a group and choosing 'Rename'.

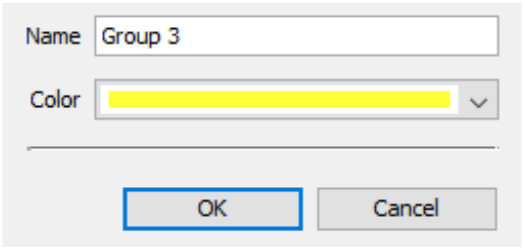


Figure 13: Adding a group.

After importing, activating and renaming all four grid gel images, the 'Light Table' should look as shown in Figure 14.

Figure 14: 'Light Table' with different groups.

2.2 Image Attributes

The next step is to set the properties of the gel images (Toolbar -> Images -> Image Attributes, Figure 15). This is essential for later warping. It is also possible to open 'Image Attributes' in the section 'Setup Project' in the 'Workflow'. The window 'Image Attributes' consists of three different categories - *Sample, Gel* and *Channel* - to which the gels need to be assigned. Gel images are moved to their target position by holding down the mouse key. Forbidden positions are marked by a black circle. Correct positions turn blue.

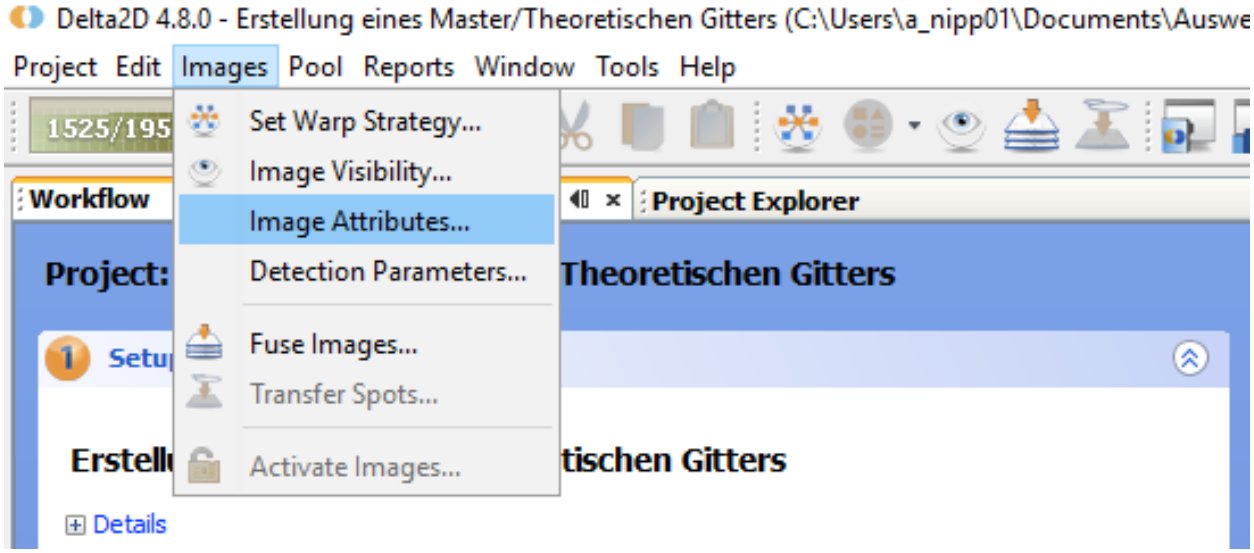


Figure 15: 'Image Attributes' in the toolbar.

2.2.1 Sample

All reference images (seen in Figure 16 as _grid_550 Cy3) are assigned to 'Sample B'. All the sample gel images (seen as _ecoli_525 Cy5) will be assigned to 'Sample A' later in the analysis. Figure 16 and 17 illustrate the assignment process.

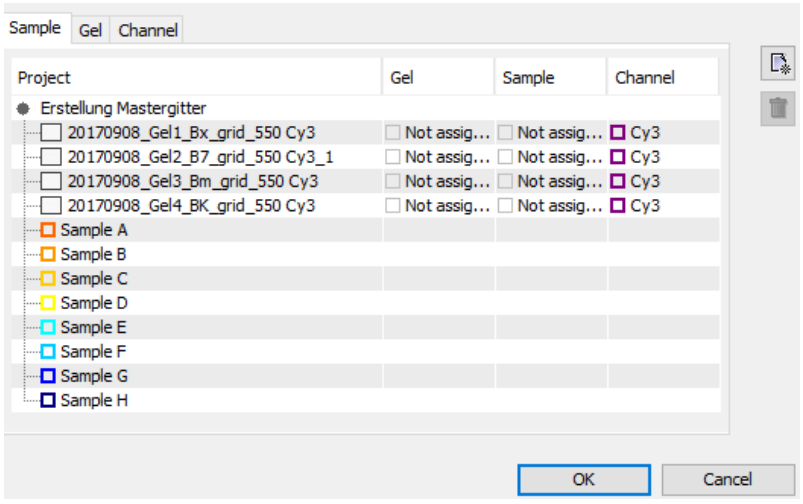


Figure 16: 'Image Attributes' – 'Sample', before assignment.

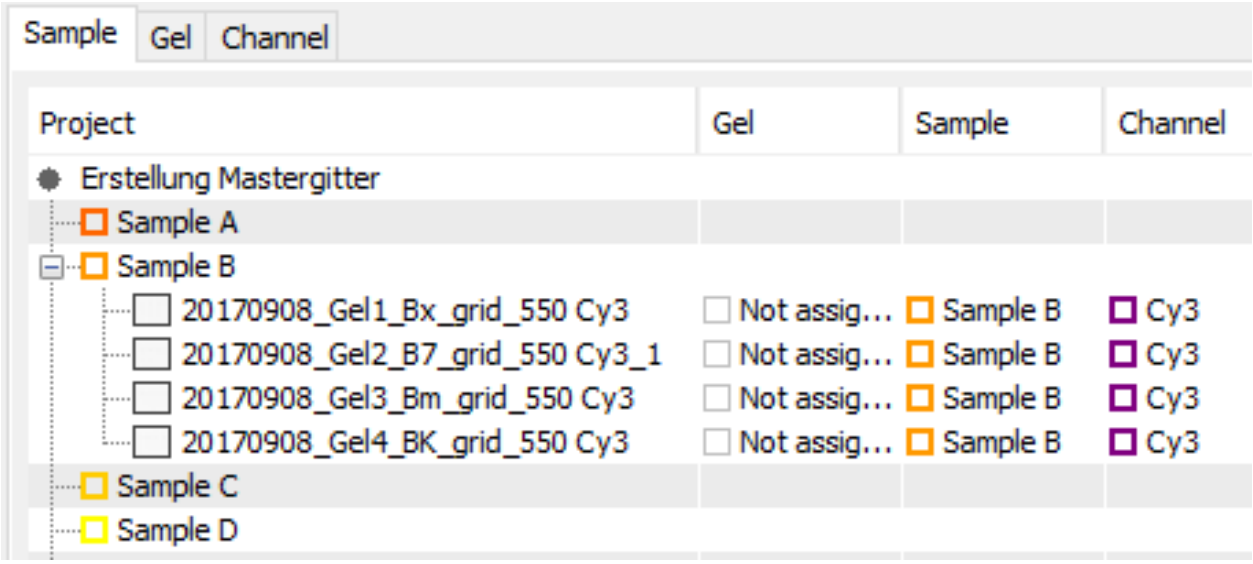


Figure 17: 'Image Attributes' – 'Sample', after assignment.

2.2.2 Gel

The most important assignment for later warping is the sorting in 'Gel' (Figures 18, 19). It is essential that gel images, which derive from one experimental gel, are also assigned to one virtual gel in Delta2D. Sample gel images will be sorted to their corresponding reference grid during import.

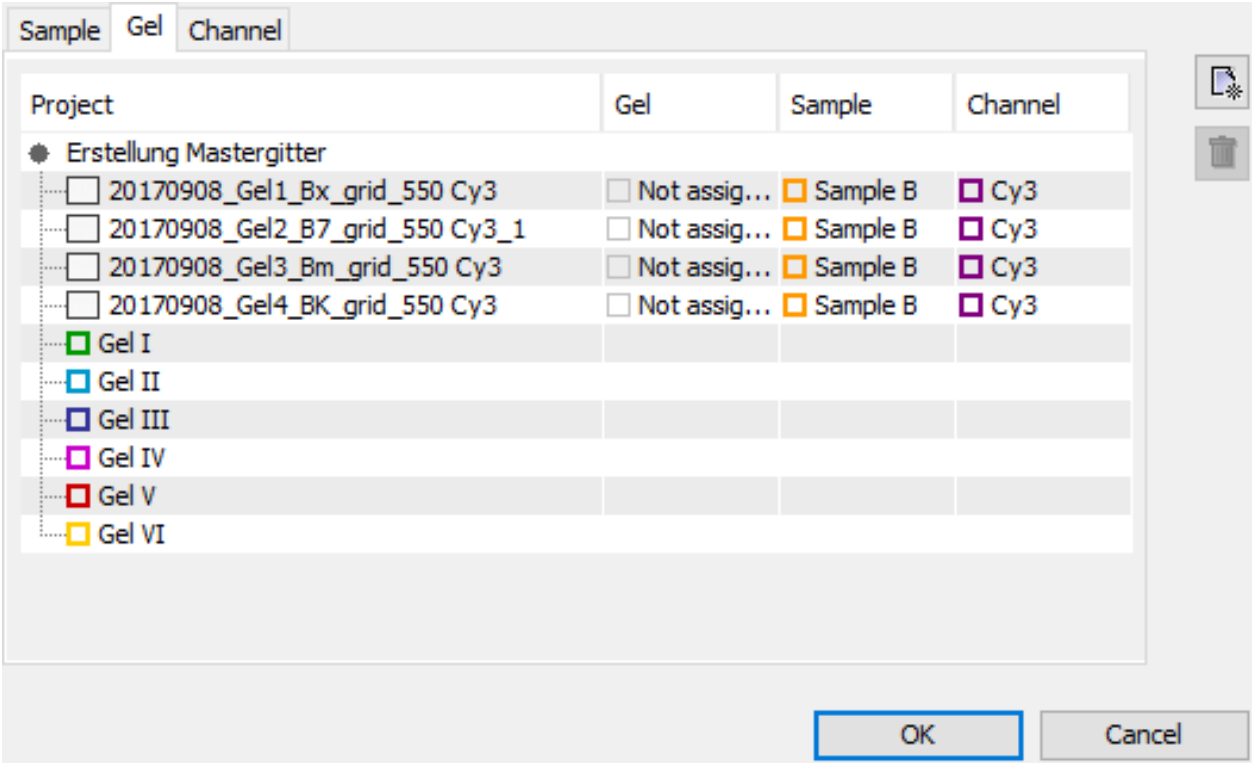


Figure 18: 'Image Attributes' – 'Gel', before assignment.

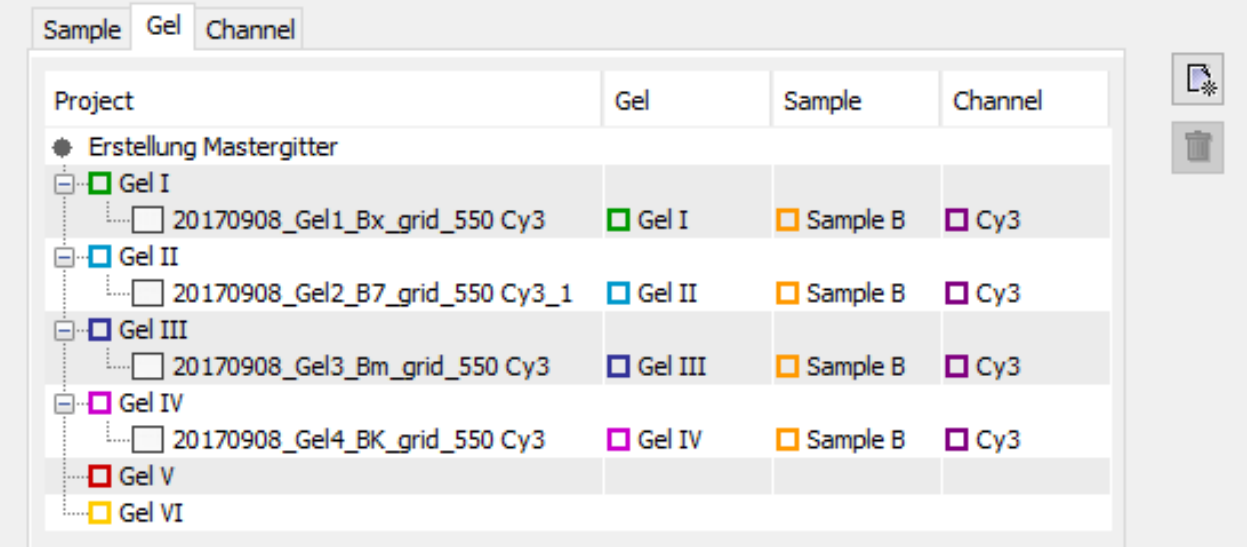


Figure 19: ‘Image Attributes’ – ‘Gel’, after assignment.

2.2.3 Channel

In the tab ‘Channel’, gels can be sorted according to the dye used. Here, the software recognizes it automatically, so nothing has to be changed (Figure 20). After closing ‘Image Attributes’, coloured squares appear on the lower left edge on the gel images, which illustrate the attributes.

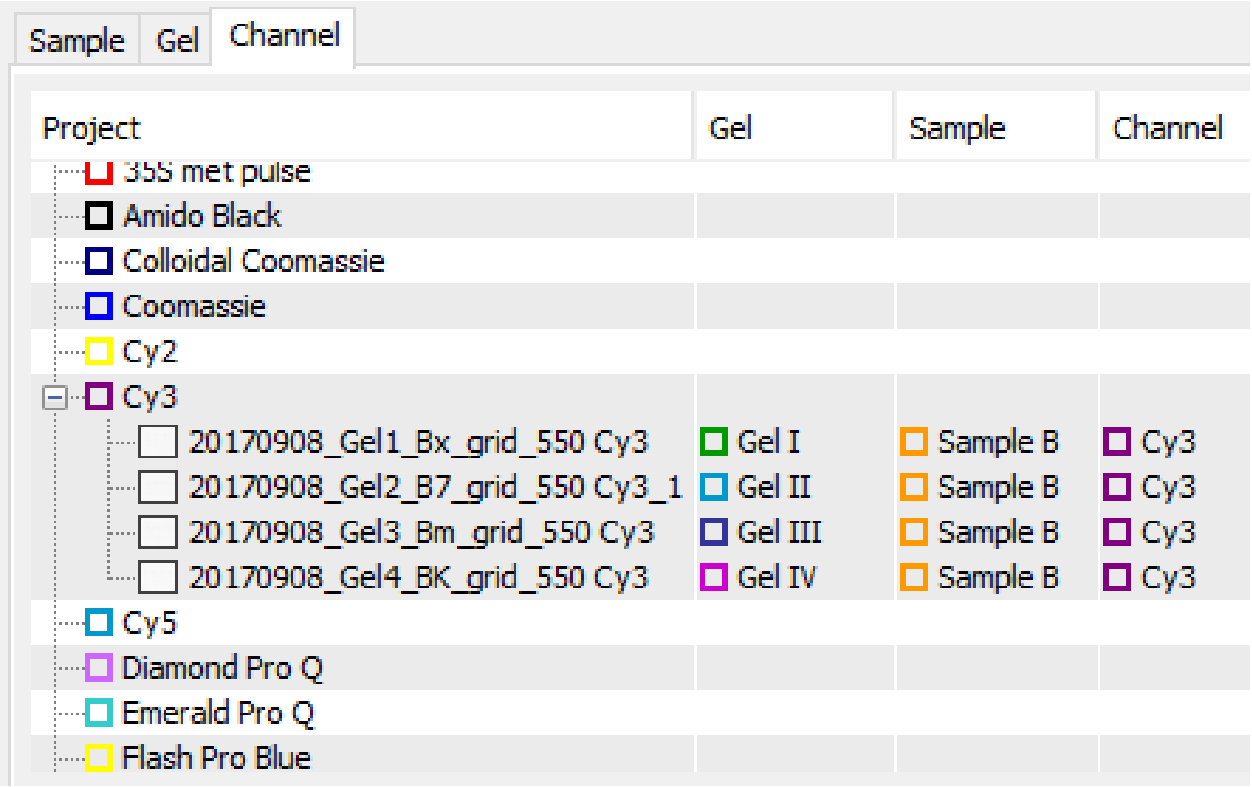


Figure 20: ‘Image Attributes’ – ‘Channel’.

3. Warping of Reference Gels

Warping deforms the gel images so that they can be virtually overlayed. Choose ‘Window’ -> ‘Warping-Setup’ (Figure 21). Switch from ‘Light Table’ to ‘Warping Setup’ (Figure 22) and on the left site of the window to the second point of the ‘Workflow’. It is important that no connections among the gel images have been created at this point.

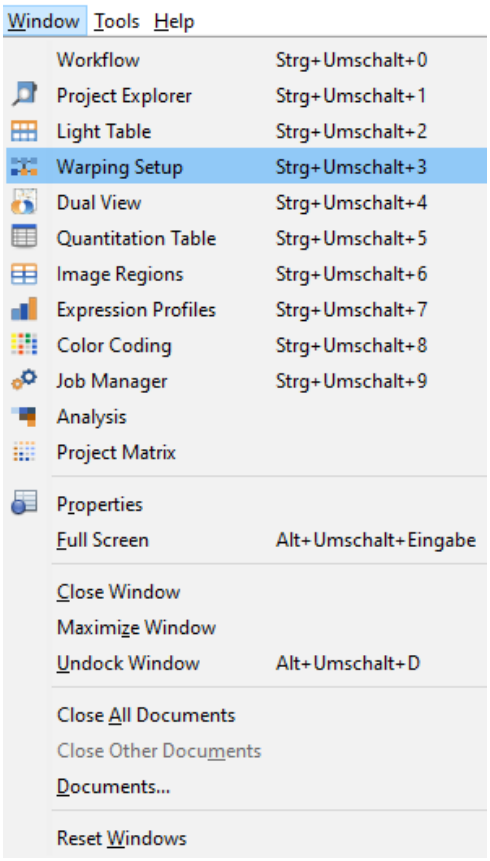


Figure 21: Start ‘Warping-Setup‘.

Figure 22: Before warping.

There are two possibilities to connect gel images for warping. A warping strategy may be selected as discussed later or gel images are overlapped manually. For instance, if the aim is a fused image of all four grid gel images, all these images must be in a warping relation. Therefore, grid 1 is defined as master and the remaining three grids are warped to it (Figure 23).

For warping, match vectors are determined for gel spots. In order to find and control match vectors on gel images, ‘Dual View’ (Figure 24) must be activated by double-click on the symbol .

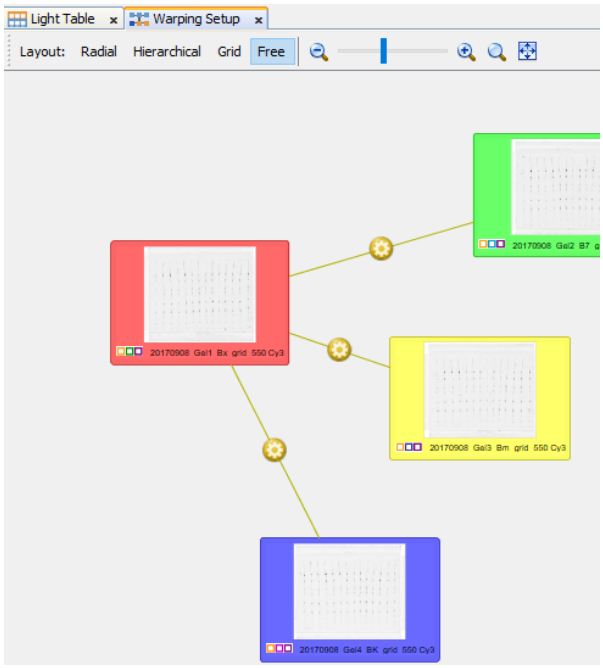


Figure 23: Warping relations between grids.

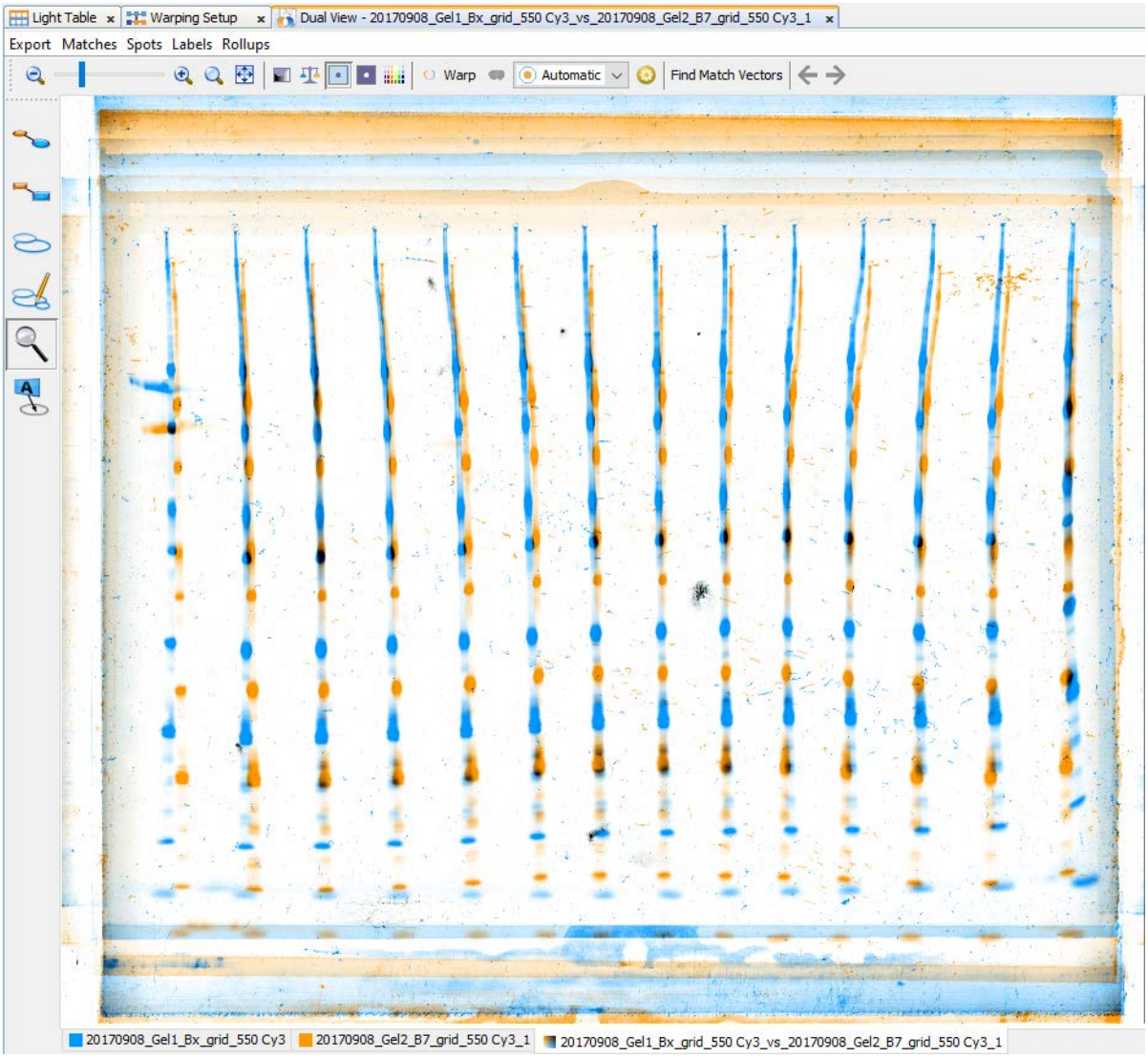


Figure 24: 'Dual View' before warping.

'Find match vectors' (Figure 25) suggests match vectors automatically. In Figure 26 the result is displayed. Unwanted match vectors can be deleted by using  and right-click / 'Delete'. To create new match vectors, the orange spot needs to be selected followed by the corresponding blue spot.

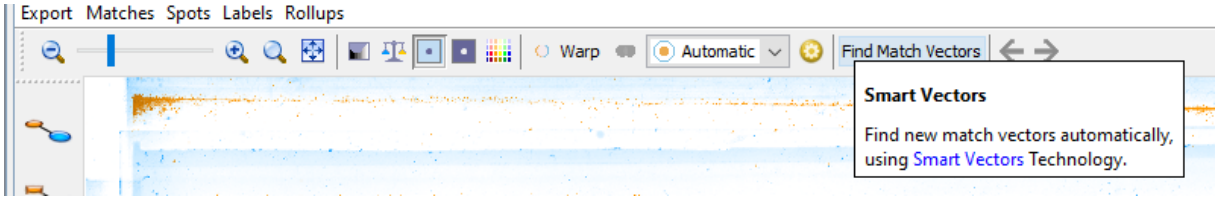


Figure 25: Toolbar 'Find Match Vector'.

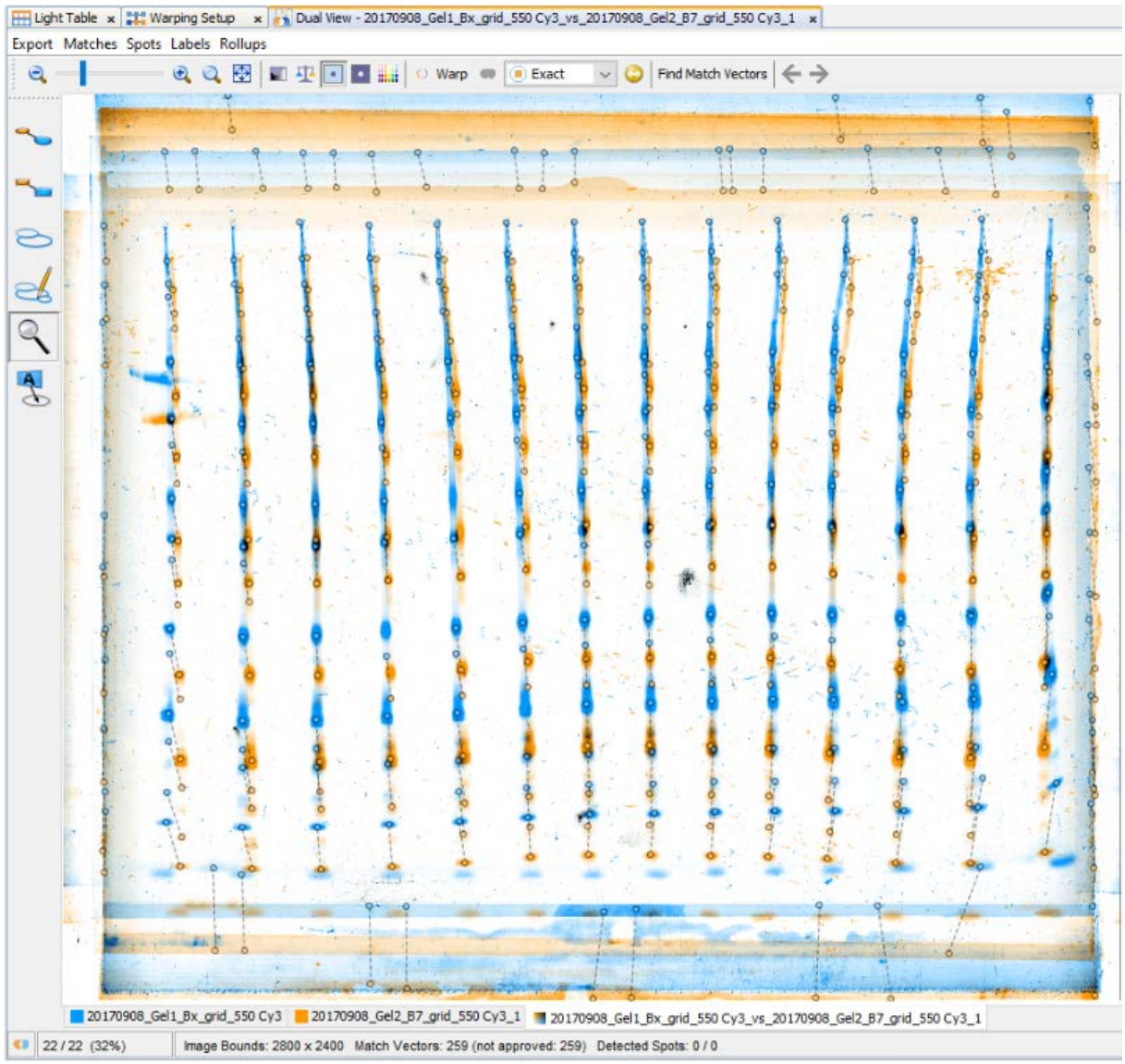


Figure 26: 'Dual View' with match vectors.

In Figure 28, the corrected match vectors are shown. A dashed line indicates that the match vector was not approved yet. A continuous line represents manually added vectors which are thus approved. It is possible to approve all vectors in the 'Workflow' (Figure 27). If all match vectors are correct, the button Warp in the toolbar has to be activated. Both gel images are warped according to these match vectors.

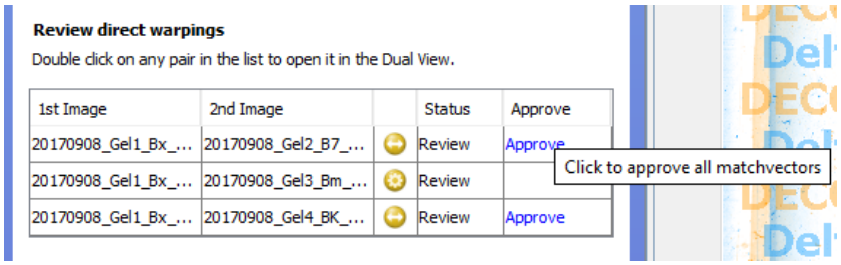


Figure 27: Match vector set in 'Approved'.

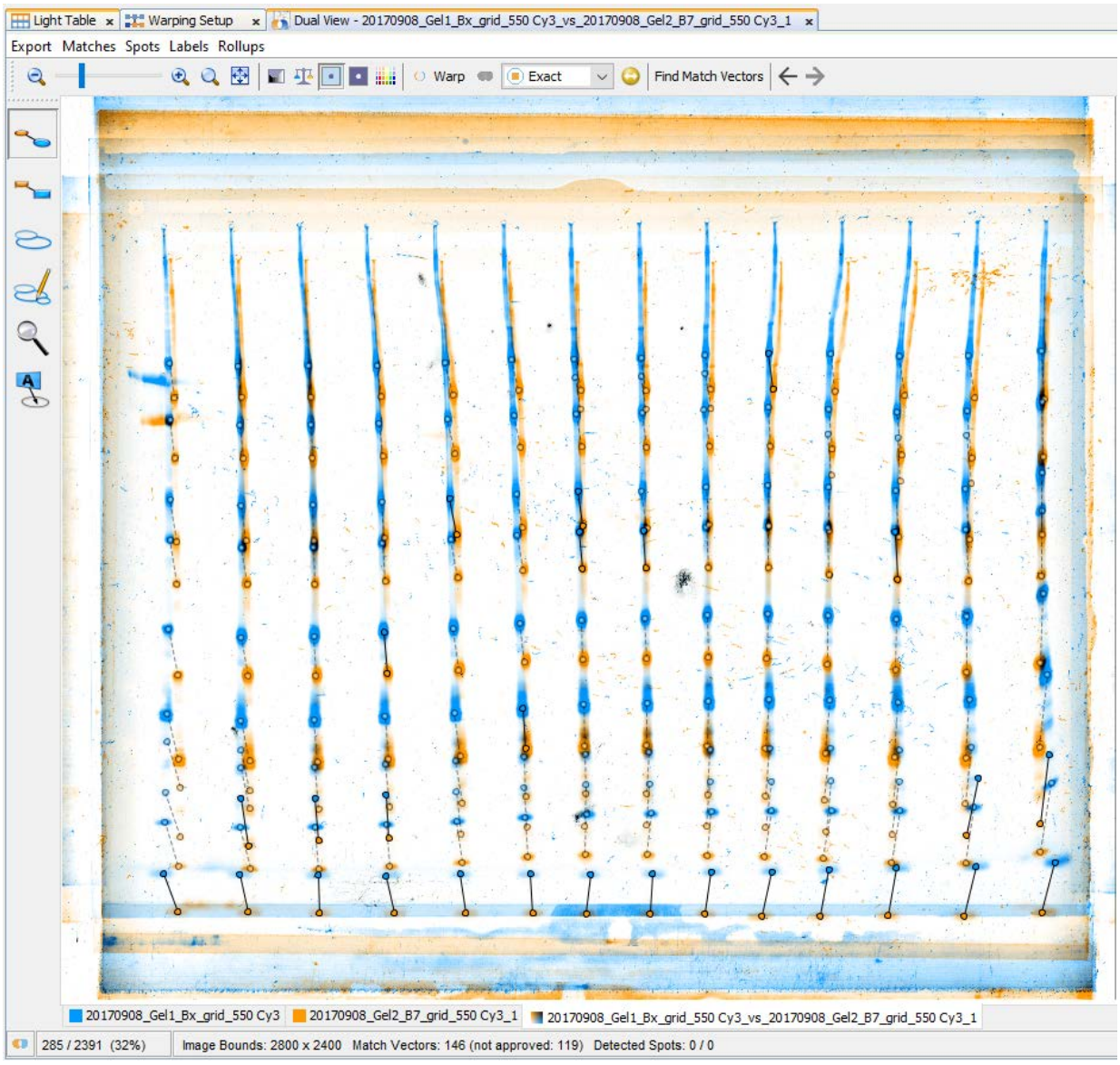


Figure 28: 'Dual View' with corrected match vectors.

The warping was successful when all blue or orange spots were replaced by black (Figure 29). If this is not the case (Figure 30), the image must be unwarped to change the match vectors. With successful warping the symbol  in the warping setup changes to  . The match vectors of all remaining gel images need to be generated in the same way.

In case Delta2D is not able to find sufficient match vectors (Figure 31), they need to be drawn manually.

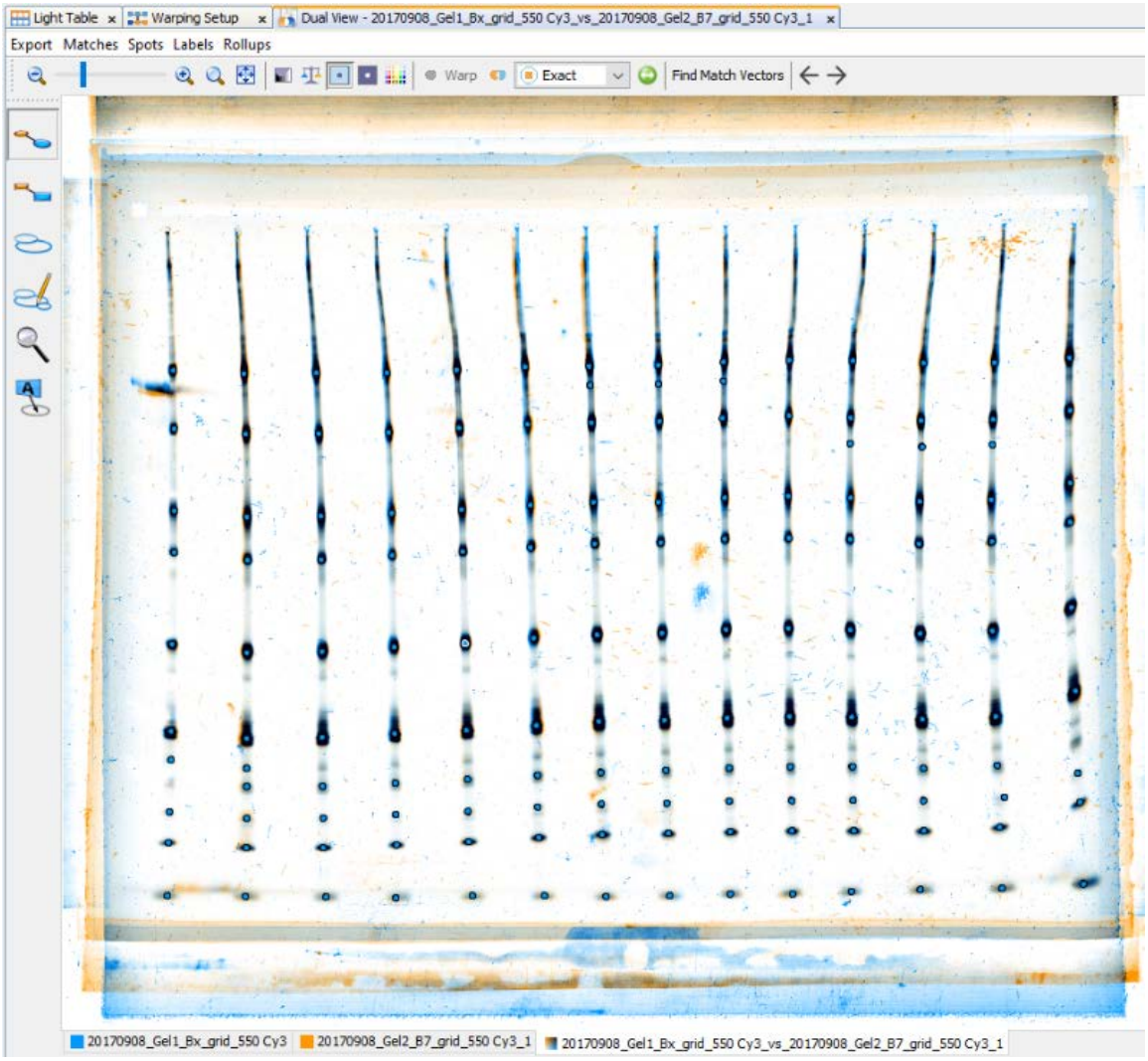


Figure 29: ‘Dual View’ after warping.

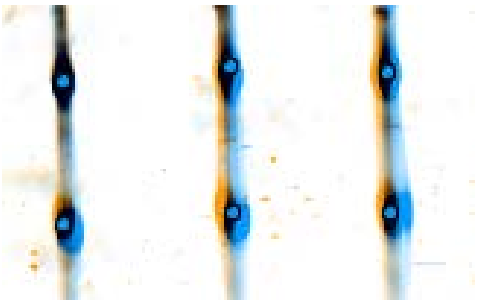


Figure 30: Example of unsuccessful warping.

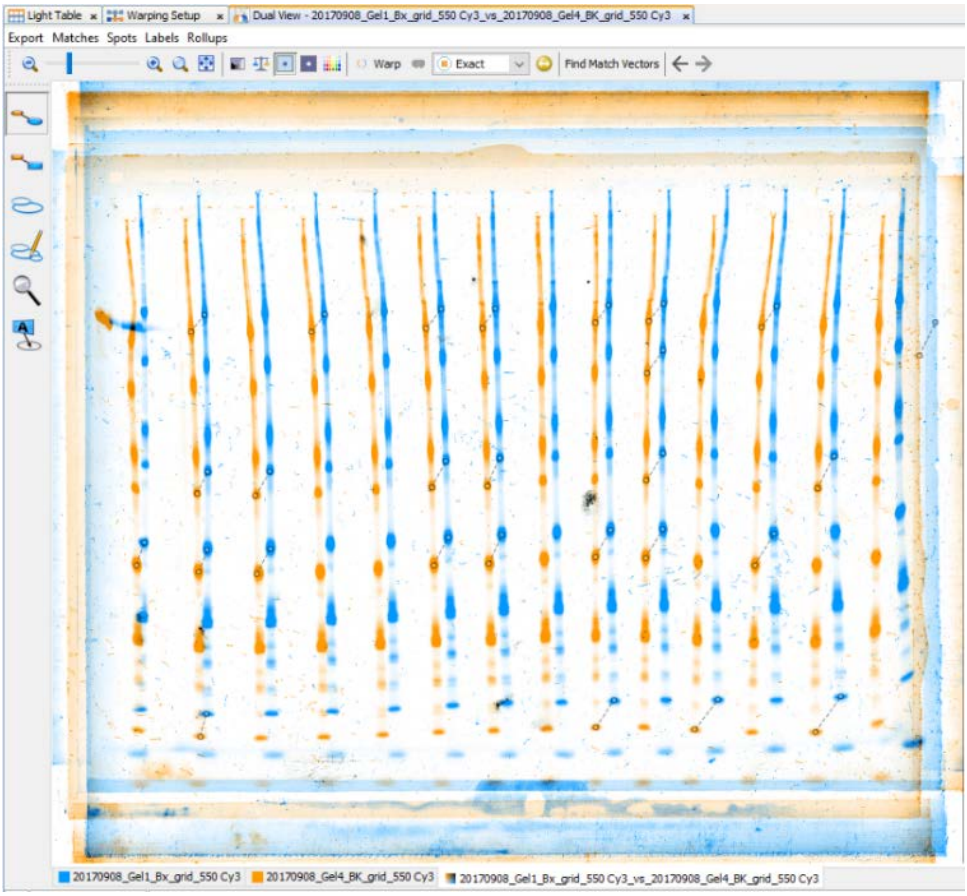
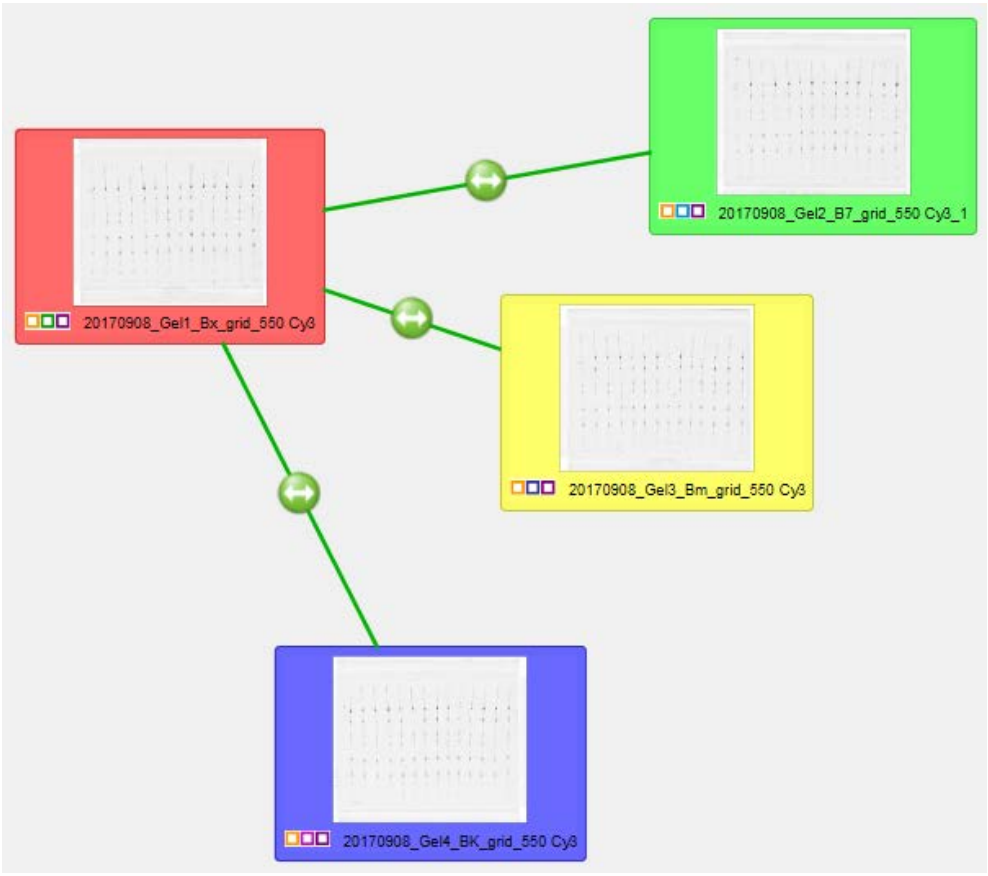


Figure 31:

'Dual View' before warping, unsufficient number of match vectors.



After executing all warpings, the warping setup should look as shown in Figure 32.

Figure 32: Warping setup after warping.

4. Creating a Fused Image

The ultimate aim is to warp all sample gels to a pregenerated master (ideal) reference grid. To that end, a fused image of all four reference grid gel images is formed (FI-RG). The fused image corresponds to above master grid 1, but it also contains information from the other grid gels. If, e.g., lane 1 was missing in grid 1, it would still appear in the fused image.

For creating a fused image, the third point 'Detect and Quantify Spots' in the 'Workflow' is opened (Figure 33). After clicking 'Fuse all images' the window 'Image Fusion' is opened (Figure 34).

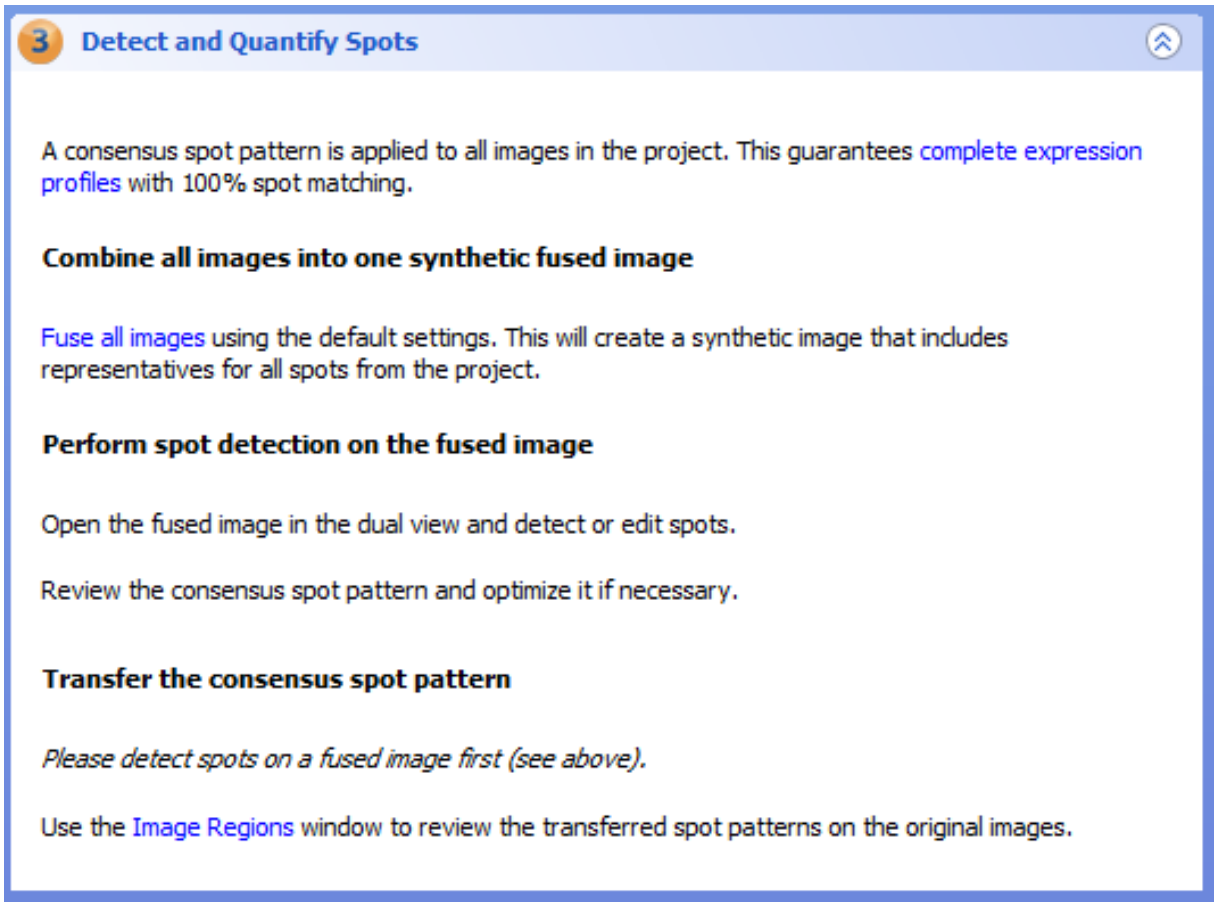


Figure 33: 'Detect and Quantify Spots' window.

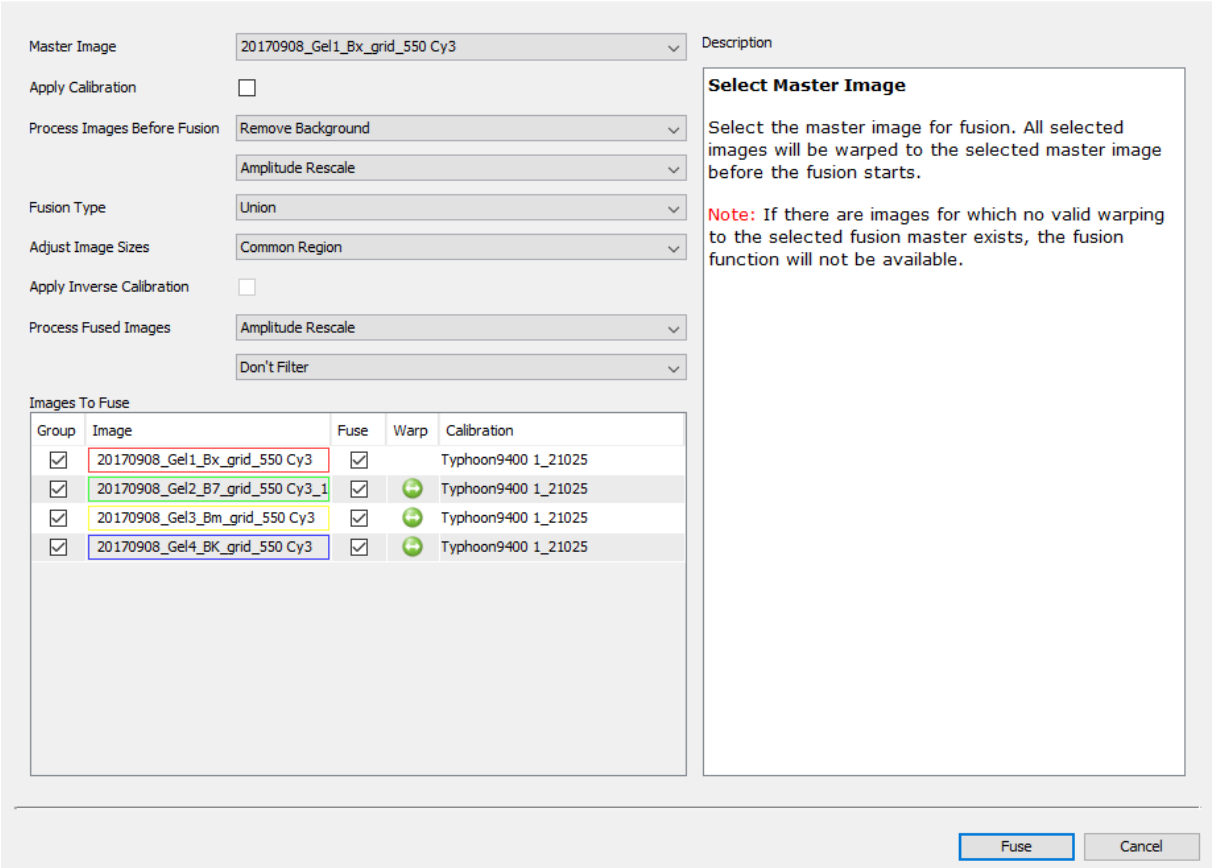


Figure 34: 'Image Fusion' window.

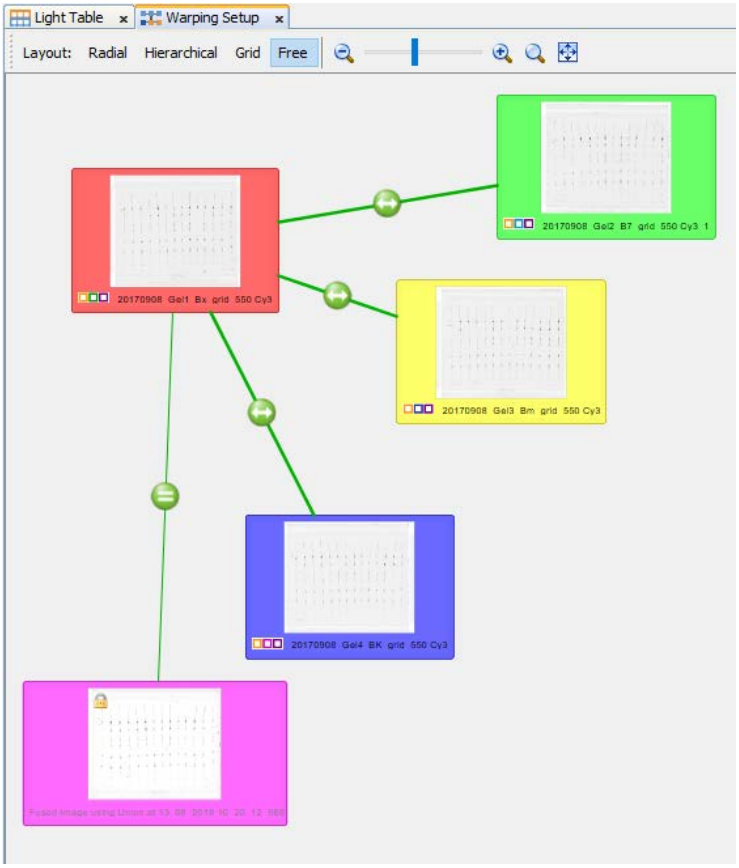
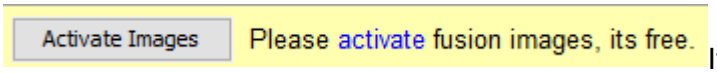


Figure 34 shows which gel images can be fused. If several gel images were imported, but not all are to be fused, some can be excluded. 'Fuse' generates a fused image.

Figure 35: Fused image in the 'Warping Setup'.

The fused image is not activated yet. 

Its activation is free of charge as long as it is activated in the same session (Figure 36). The fused image is shown in Figure 37. No duplicate spots are observed confirming successful warping.

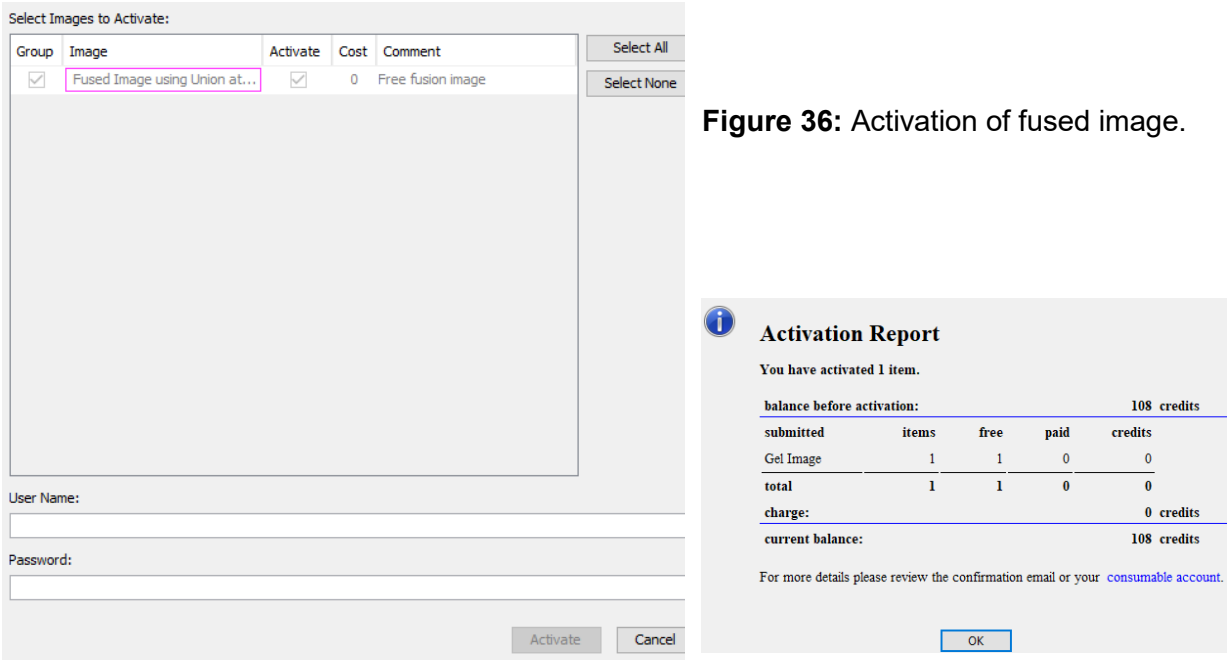


Figure 36: Activation of fused image.

Figure 37: Fused image.

5. Warping of Sample Gels

5.1 Import and Image Attributes

It is necessary to create a new project for import and warping of sample gels on the basis of the previously generated fused image (FI-RG). For creating a new project, the column ‘Project’ is opened and ‘New Project’ is chosen (Figure 38). After saving the old project, a new project page opens. Because the grid gel images are already present in the pool, they are available when the window ‘Add images’ is opened. By clicking all the gel images while pressing ‘Ctrl’, all are selected. With ‘OK’ the images are imported to the new project.

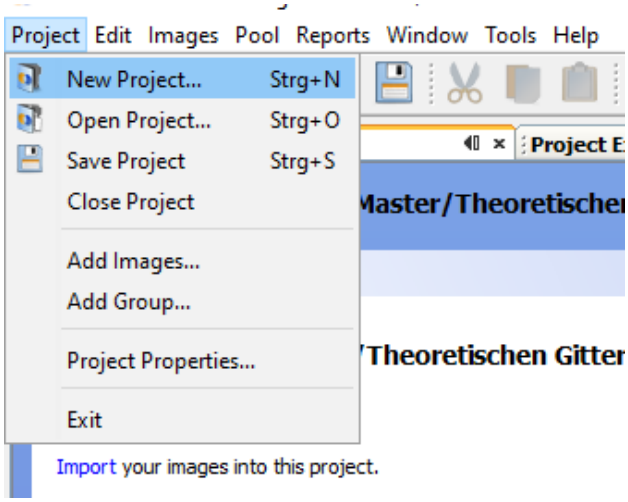


Figure 38: Creating a new project.

The sample gel images and FI-RG need to be imported and edited at ‘Import images’. The ‘Light Table’ should then look as seen in Figure 39. The newly imported images need to be assigned to ‘Sample’, ‘Gel’ and ‘Channel’ at ‘Image Attributes’ (Figures 40-42). Figure 43 shows the modified ‘Light Table’ with coloured squares after assignment.

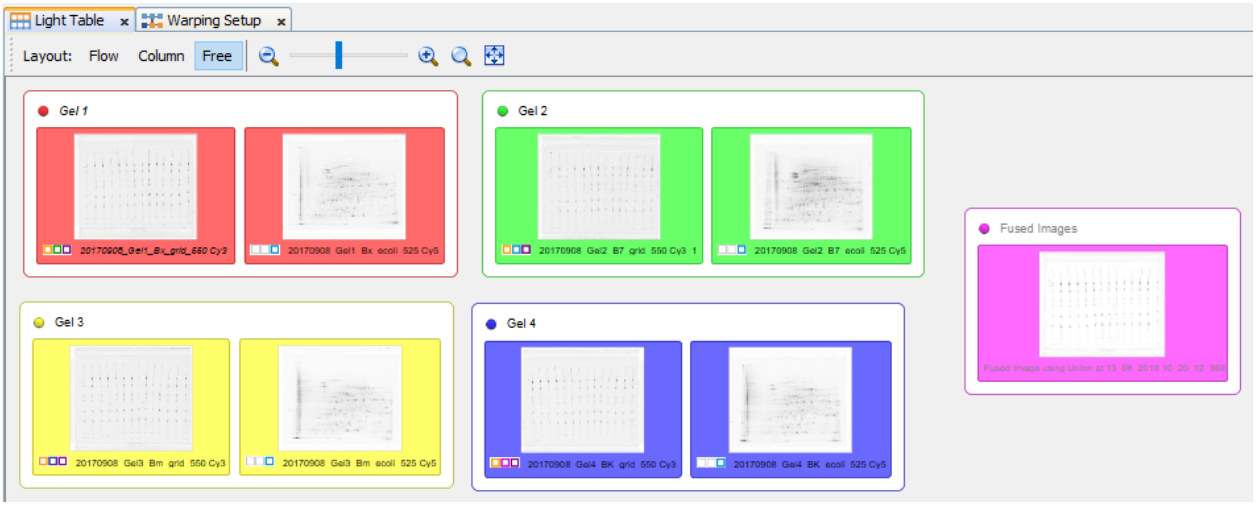


Figure 39: ‘Light Table’ with FI-RG.

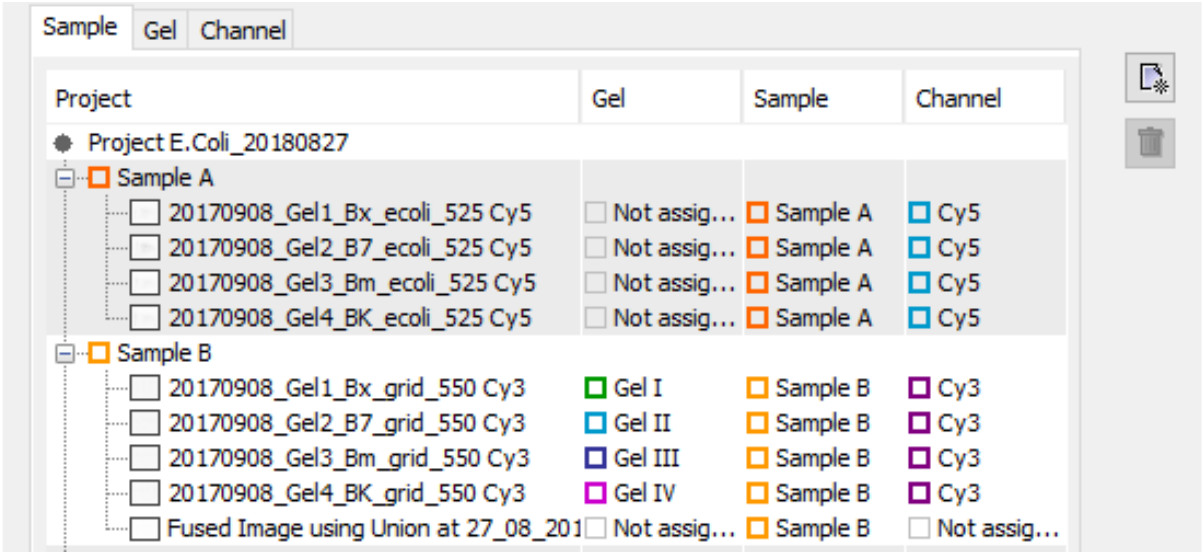


Figure 40: ‘Image Attributes’ – ‘Sample’ for sample gels and fused image.

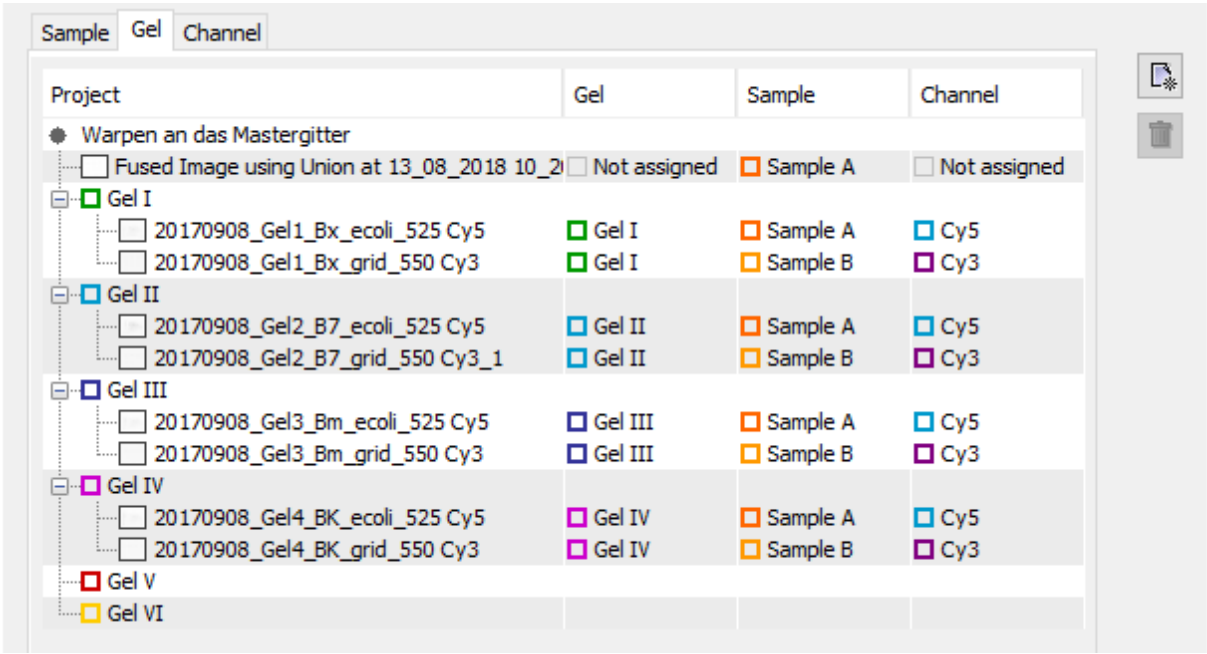


Figure 41*:* ’Image Attributes’ –‘Gel’ for sample gels and fused image.

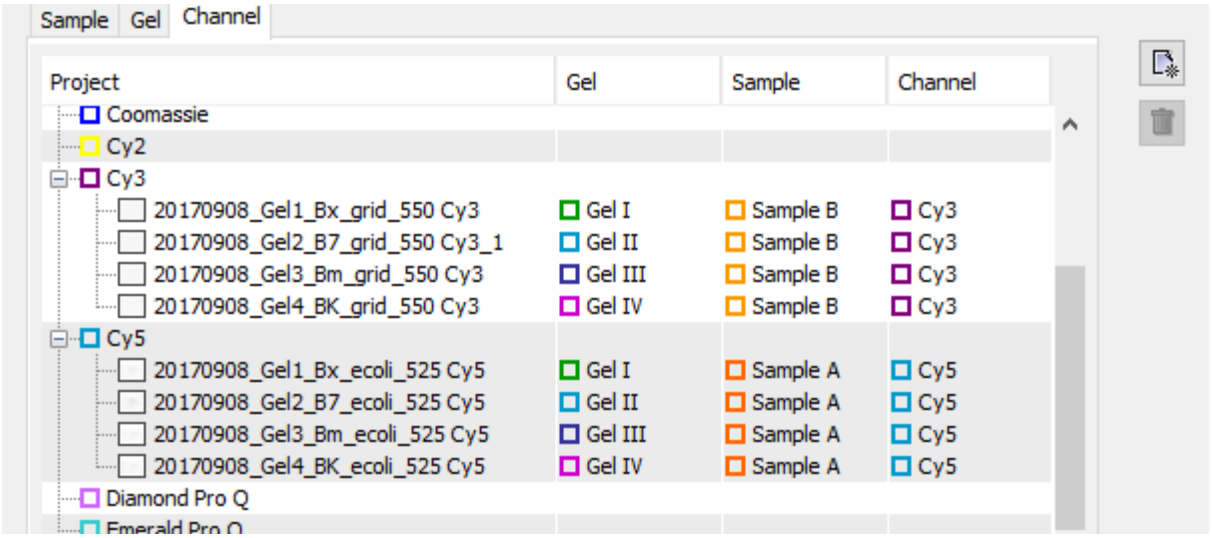


Figure 42: 'Image Attributes' – 'Channel' for sample gels and fused image.

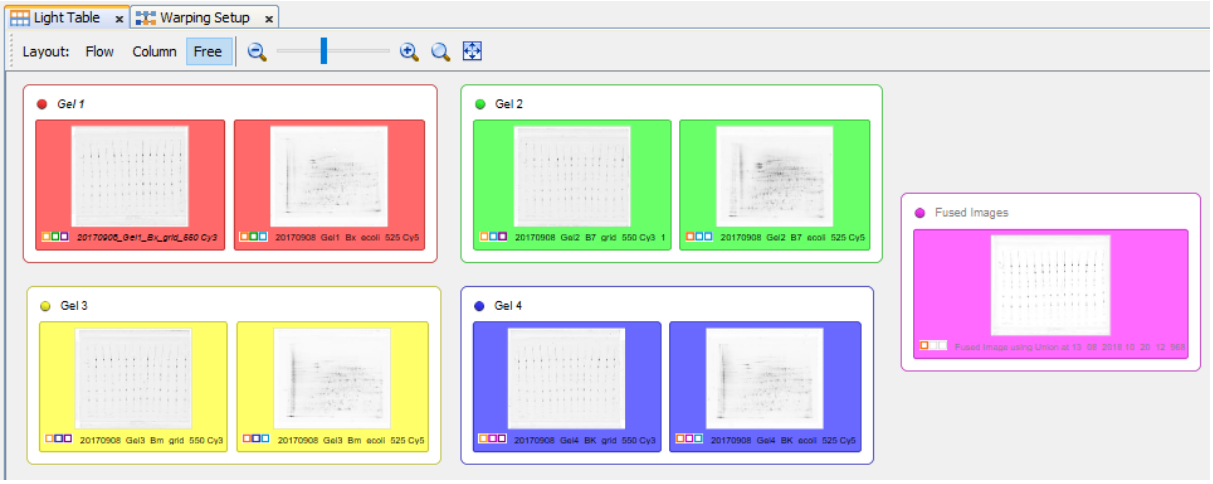


Figure 43: 'Light Table' after changing 'Image Attributes'.

5.2 Warping Strategy

The new project was created in the same pool as the first project. Therefore, the software remembers existing warping relations (Figure 44). All warping relations between grid gel 1 and the other gels must be deleted (Figure 45). Now warping relations using 'Warping Strategy' via the second point of the workflow 'Warp Images' can be generated. The suitable warping strategy is the 'Group Warping Strategy' (Figure 46).

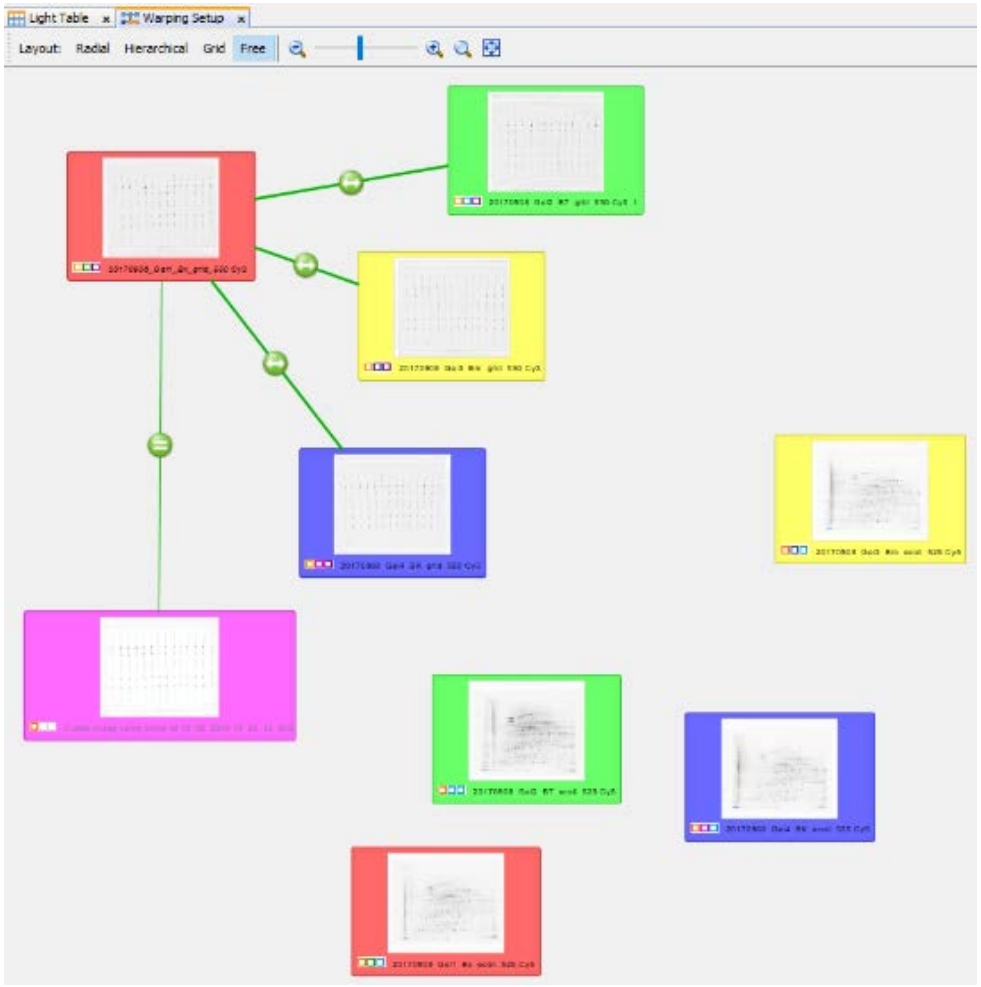


Figure 44:

'Warping Setup' with sample gels and FI-RG before application of 'Warping Strategy'.

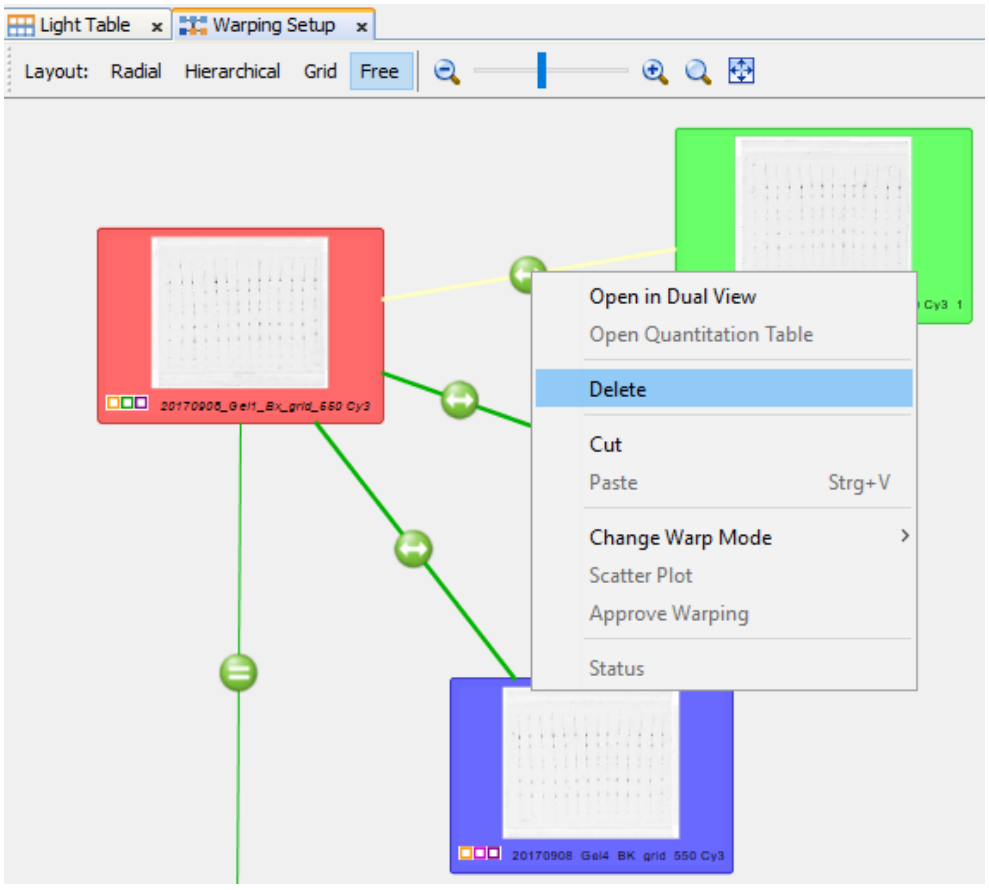


Figure 45: Removal of warping relations.

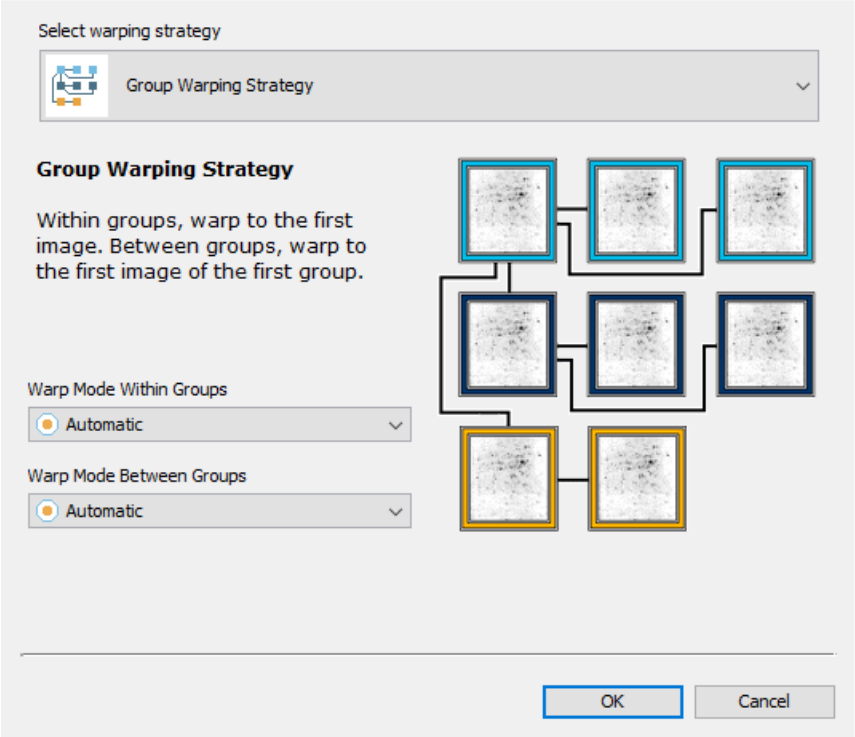


Figure 46: Group Warping Strategy.

By assigning gel images to experiments, the relation between images is recognized. The warping strategy in Figure 47 does not correspond to the correct warping relations yet.

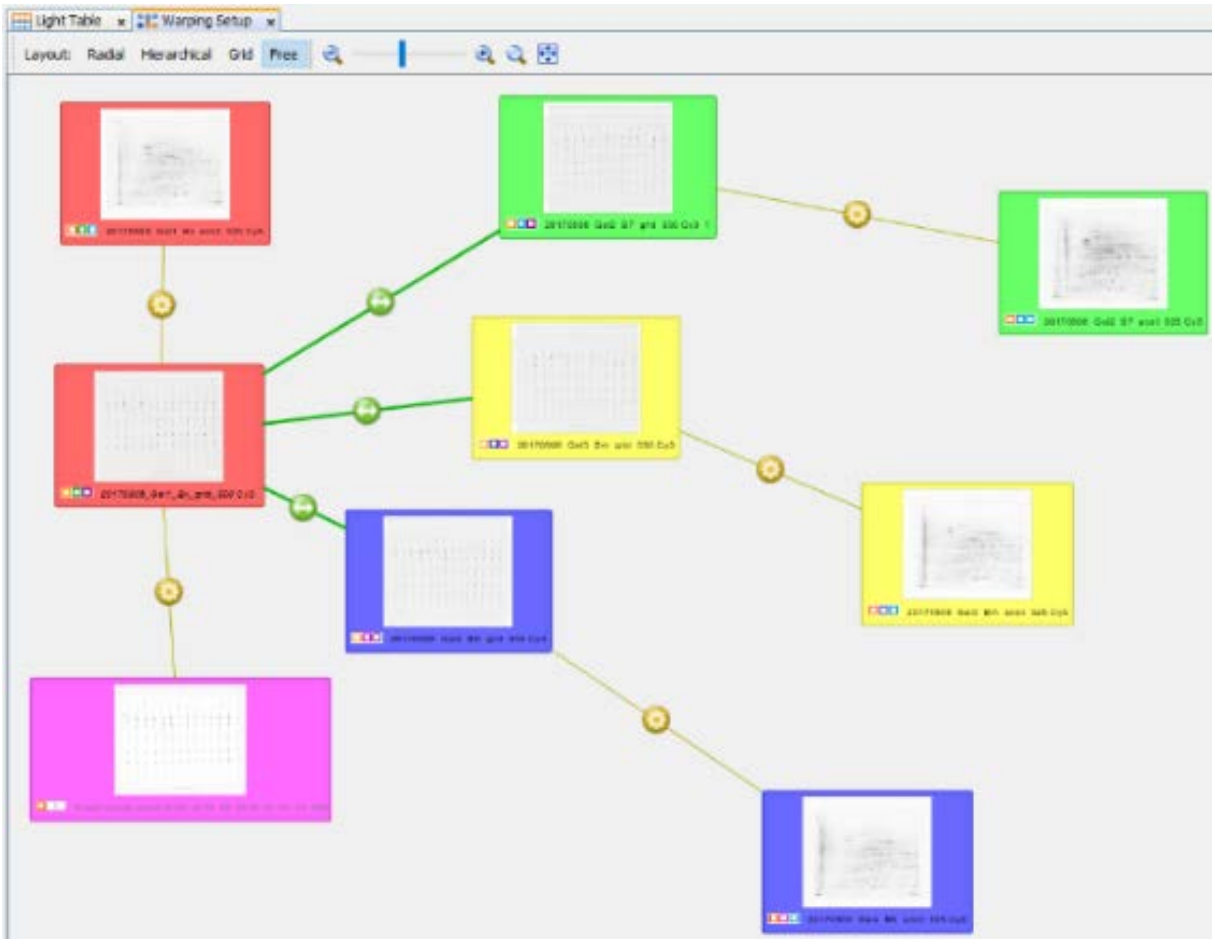


Figure 47: 'Warping-Setup' after application of 'Group Warping Strategy'.

Warping relations between the grid images and grid image 1 must be deleted. Subsequently, the grid images need to be warped to FI-RG, which now takes the role grid gel 1 had in the first project. Figure 48 shows the unmodified warping setup with FI-RG as the master grid.

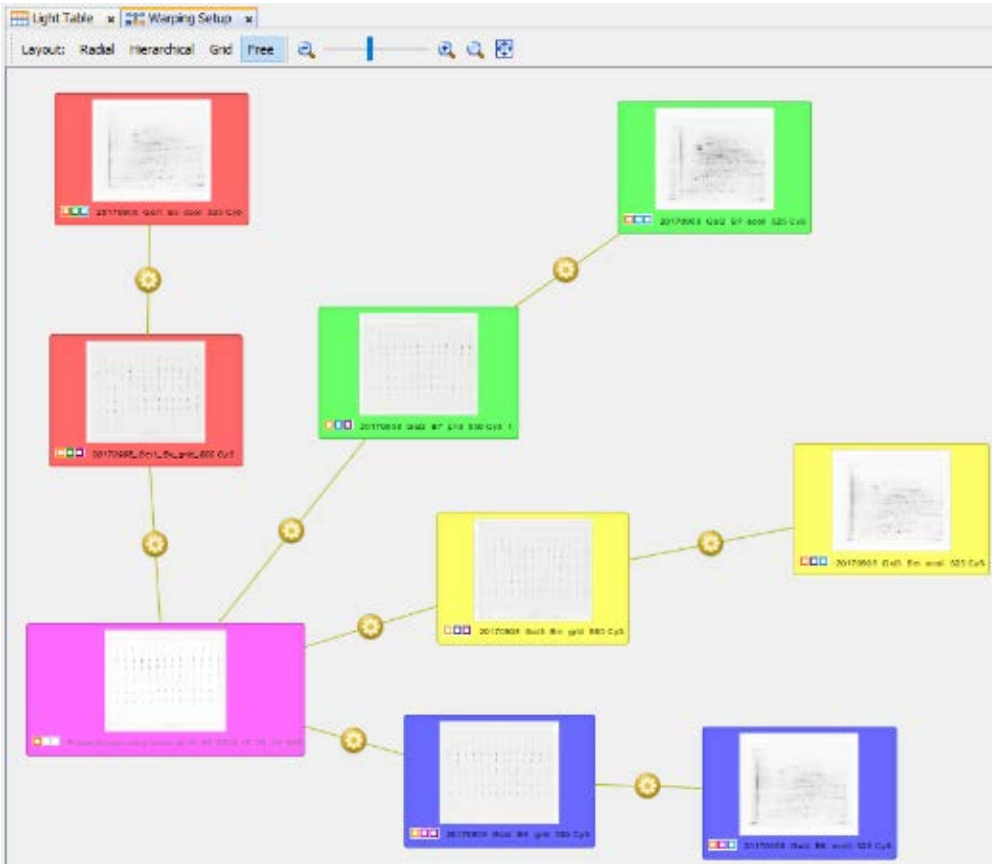


Figure 48: 'Warping Setup' with FI-RG as master gel.

The next step is to determine the match vectors between the grid gel images and FI-RG (see section 3 'Warping of Reference Gels'). The warping mode between FI-RG and grid gel 1 is set to 'Identical' (Figure 49), because grid gel 1 was used as master gel for creating FI-RG. All sample gels are also set to warp mode 'Identical' with their corresponding reference grid. This is necessary, because the software is not able to warp grid and sample as the analytes (standard vs. sample) are very different. By use of warp mode 'Identical' and the previous assignment to the same gel, the sample image is warped according to match vectors drawn between FI-RG and their reference grid image.

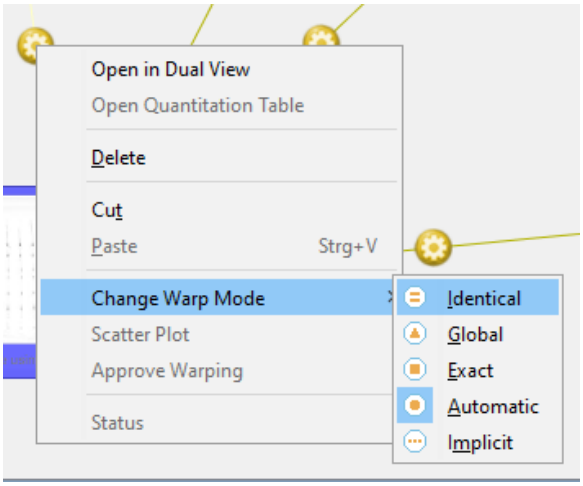


Figure 49: Change of warp modes.

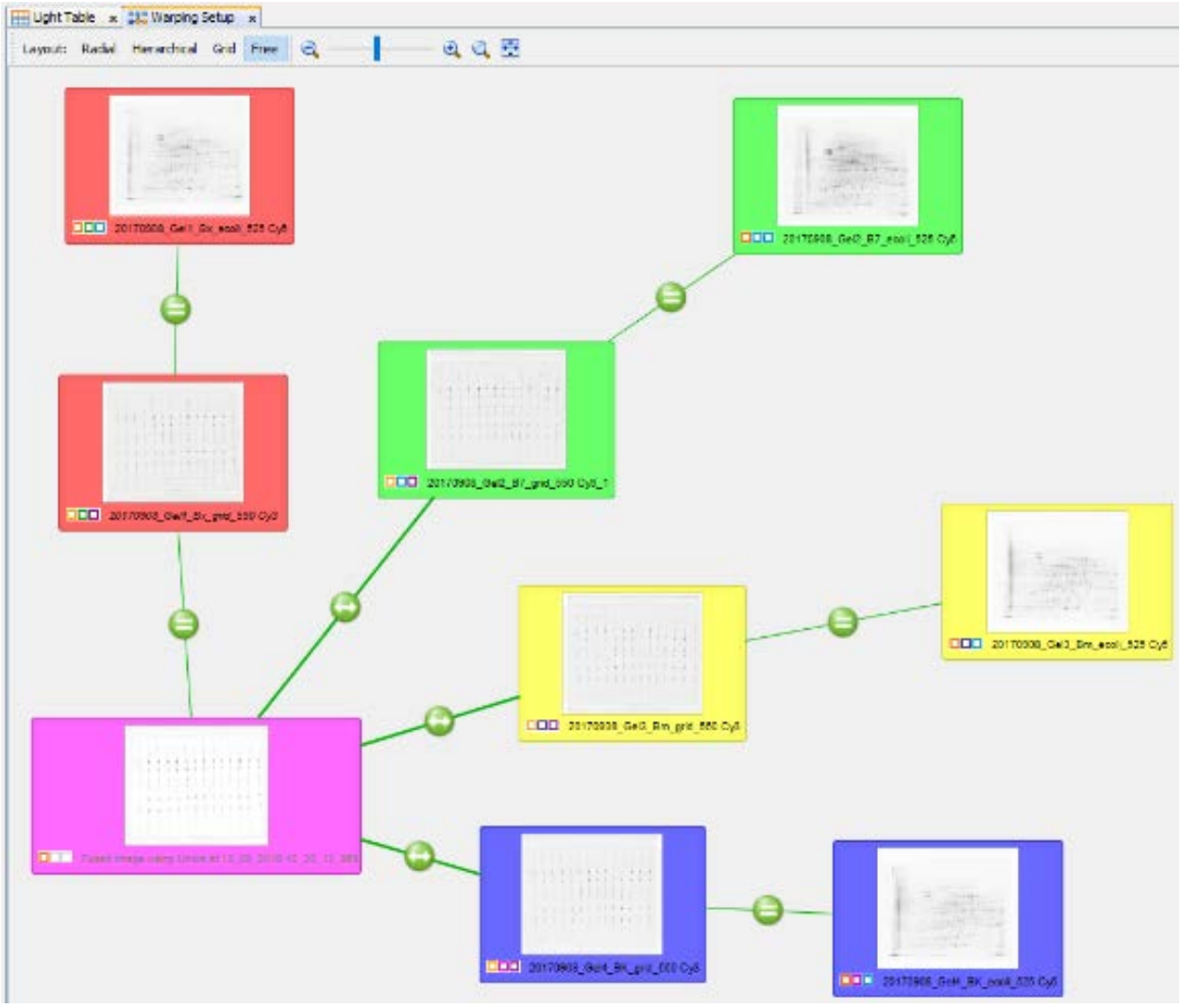


Figure 50: Warping setup with FI-RG as master gel, correct warping relations.

5.3 Visualization of Warping Results

In order to double-check the warping results, Figures 51-53 are compared. The ‘Dual View’ of a grid and a sample is generated by selecting the grid and by pressing the ‘Ctrl’ button while clicking on the sample image. When both gel images are selected, the symbol  in the toolbar is activated. Figure 51 shows the ‘Dual View’ of sample 2 and FI-RG before warping, Figure 52 after warping. The comparison of this view with the ‘Dual View’ of sample 1 and FI-RG in Figure 53 illustrates that the postion of the sample spots is clearly improved with respect to FI-RG.

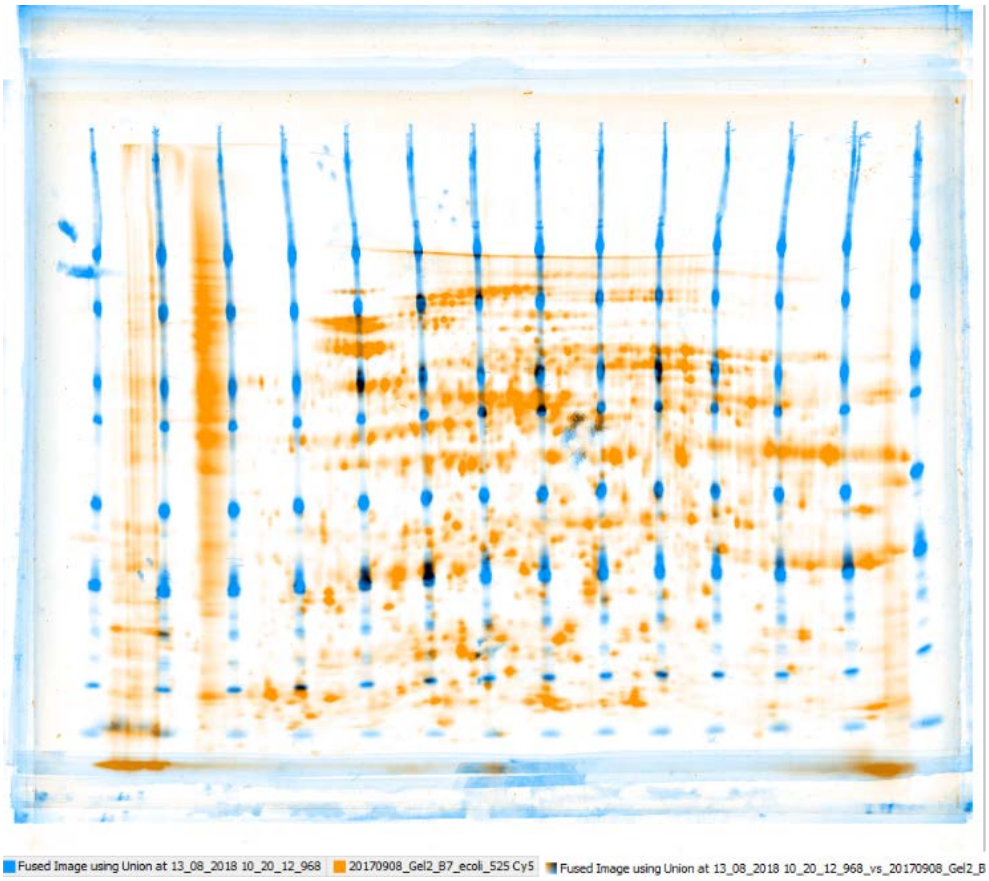


Figure 51:

'Dual View' FI-RG vs. sample gel 2 before warping.

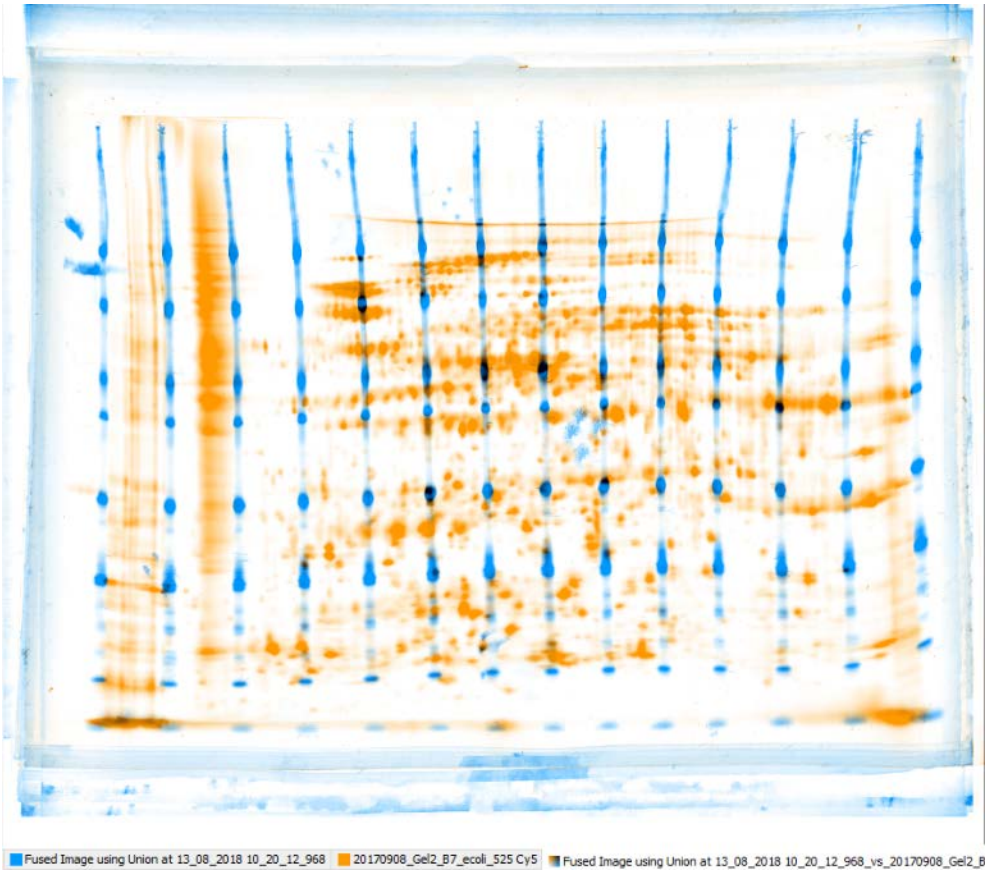


Figure 52:

'Dual View' FI-RG vs. sample gel 2 after warping.

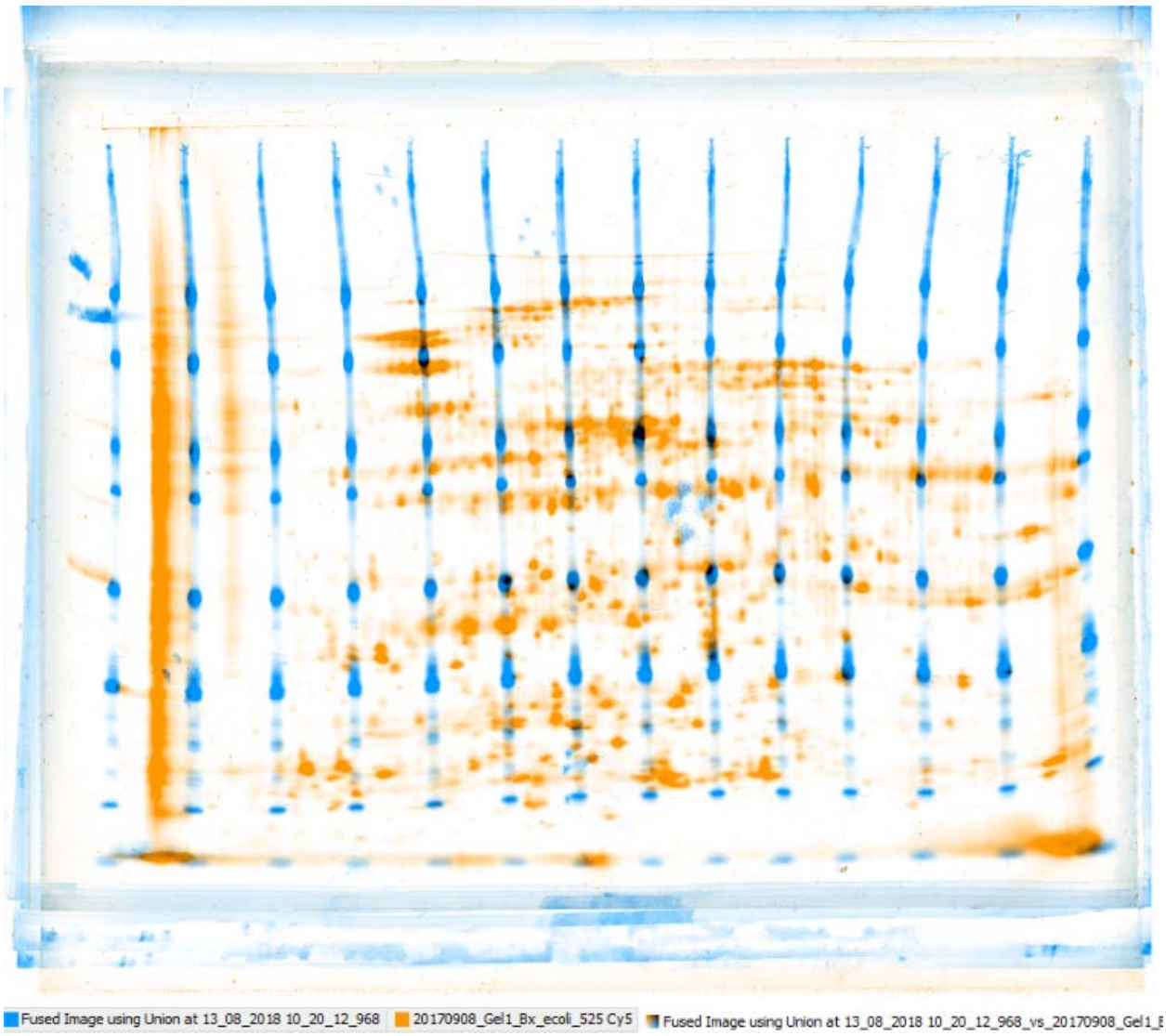


Figure 53: 'Dual View' FI-RG vs. sample gel 1 after warping.

6. Export of Coordinates

Delta2D typically only exports x- and y-spot coordinates for unwarped raw data. In order to export corrected coordinates after the warping process, more effort is required.

6.1 Export of Raw Data Coordinates

For coordinates of the sample gel, the image needs to be opened and spots need to be detected. For that, the column ‘Spots’ must be chosen in the toolbar (Figure 54).

The first point ‘Detect Spots on 20170908_Gel2_B7_ecoli_525 Cy5…’ is chosen. Afterwards the detected spots appear on the gel (Figure 55).

With the ‘Spot Selection Tool’ it is possible to delete or hide irrelevant and mark important spots (Figure 56). Additionally, spots can be labeled using the ‘Label Tool’, e.g. with a number or a name (Figure 57). This is especially helpful for the retrieval of certain spots in the quantitation table explained below.

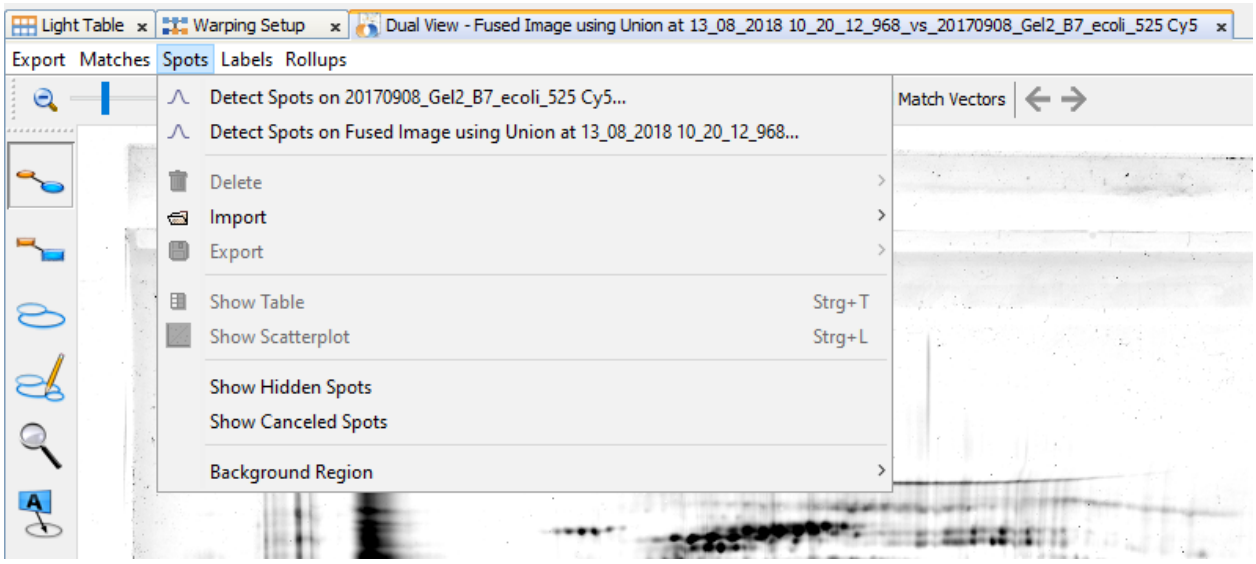


Figure 54: Spot detection toolbar.

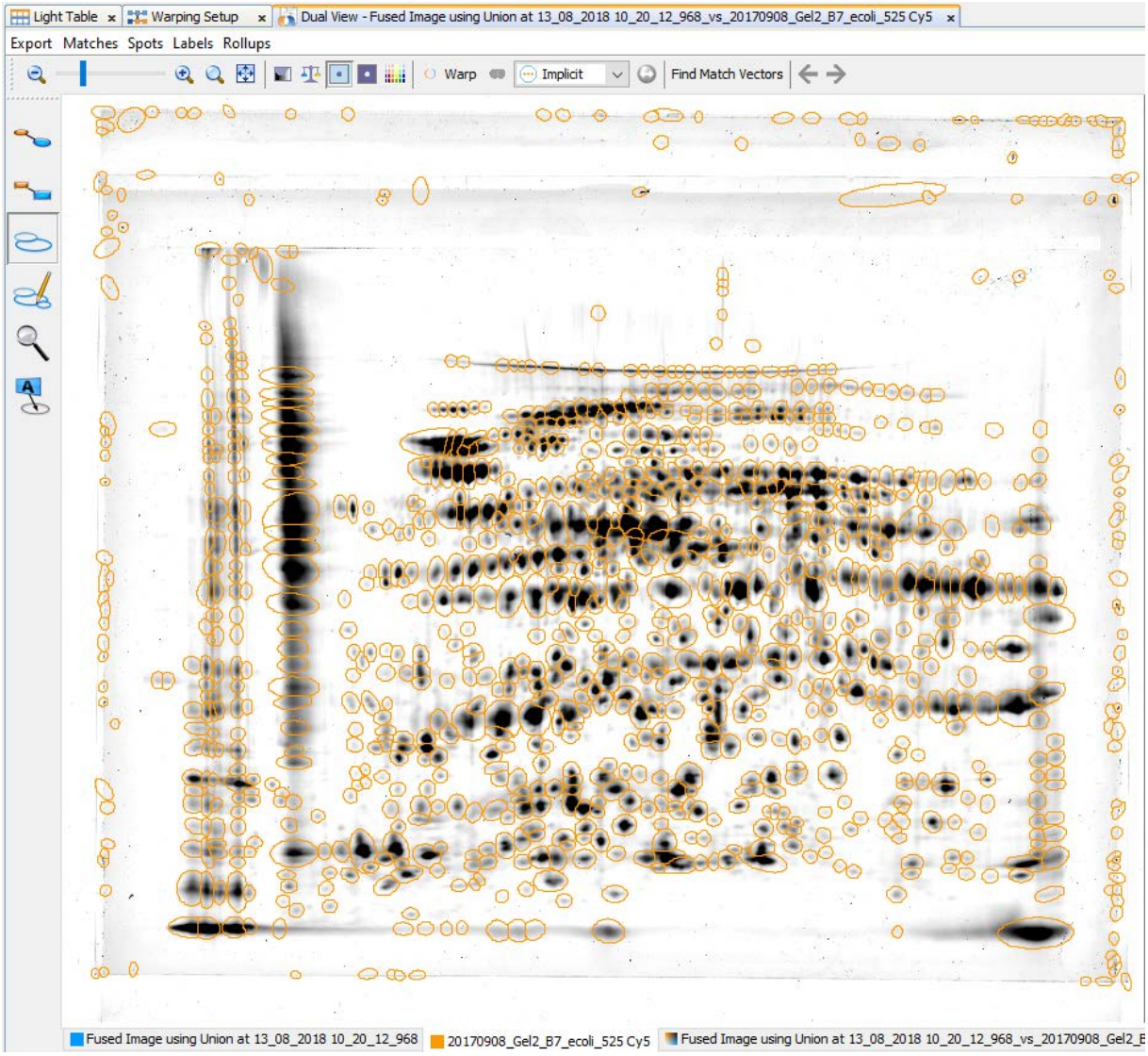


Figure 55: Detected spots on sample gel 2.

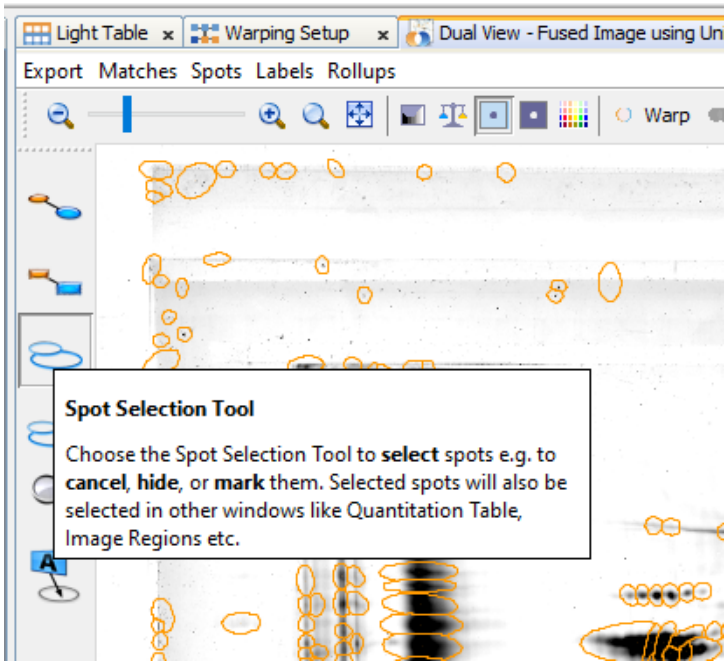


Figure 56: 'Spot Selection Tool'.

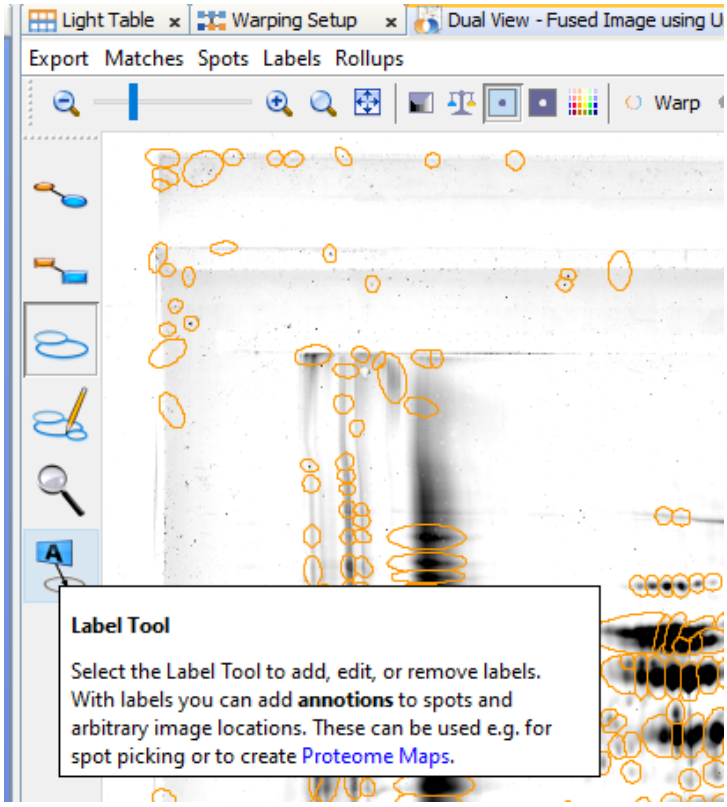
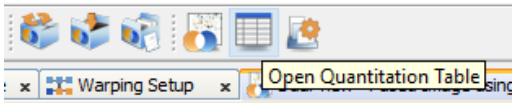


Figure 57: 'Label Tool'.

To obtain the coordinates for each gel, 'Quantitation Table' in the upper toolbar is opened



(Figure 58).

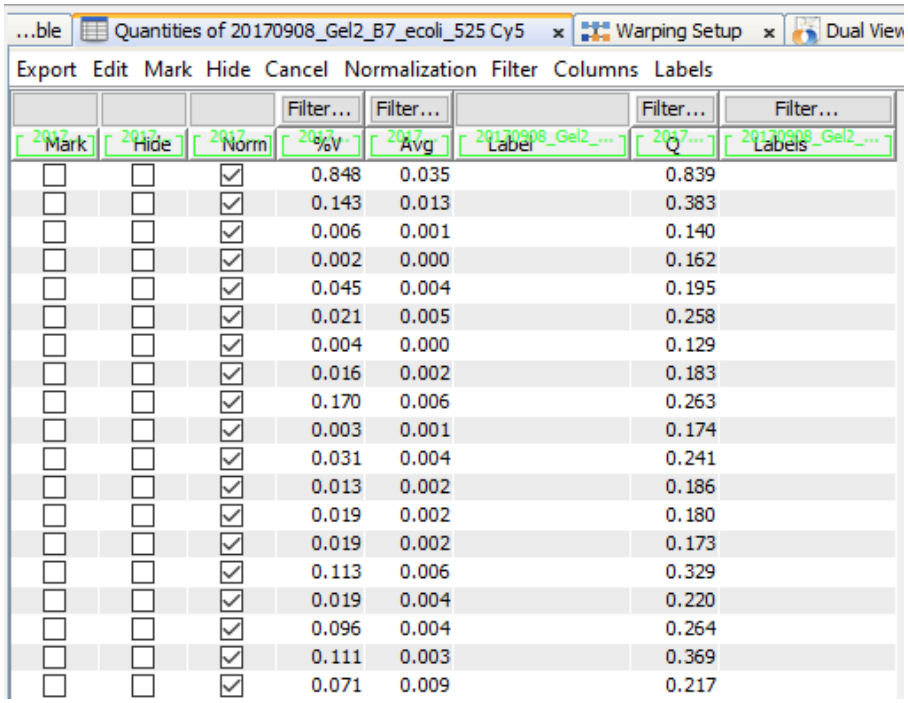


Figure 58:

Quantitation table.

In ‘Columns’, parameters can be (de)selected (Figure 59). Because the spots were not labeled in this example, the spot ID column can be chosen (Figure 60). It is possible to export the data to Excel. This procedure only works with activated images. For that purpose, the column ‘Export’ is chosen and afterwards ‘Export Spreadsheet’ (Figure 61-63).

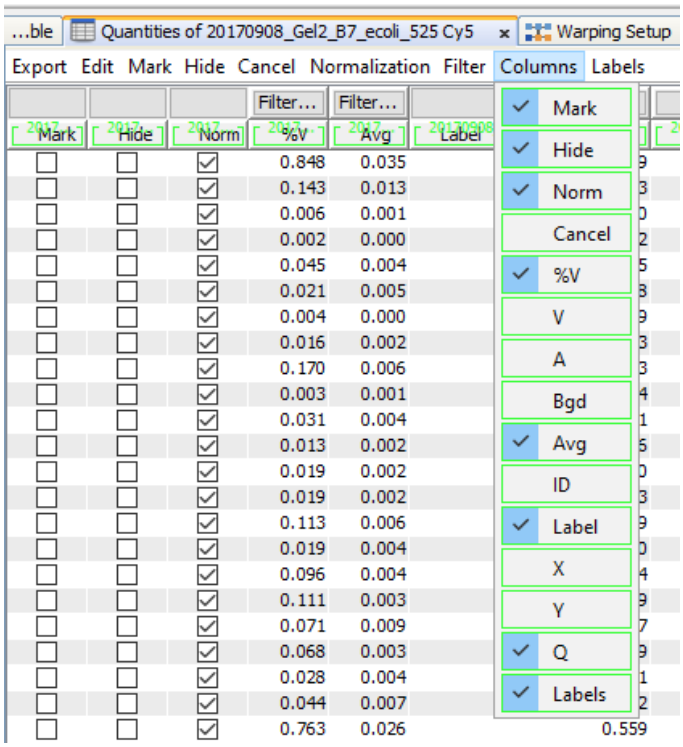


Figure 59: Selection of columns in the quantitation table.

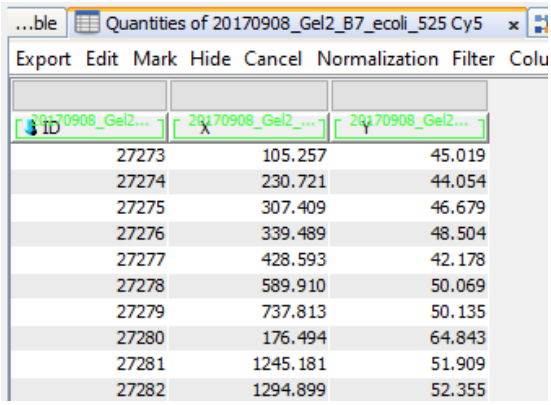


Figure 60: Extract quantitation table with coordinates and ID

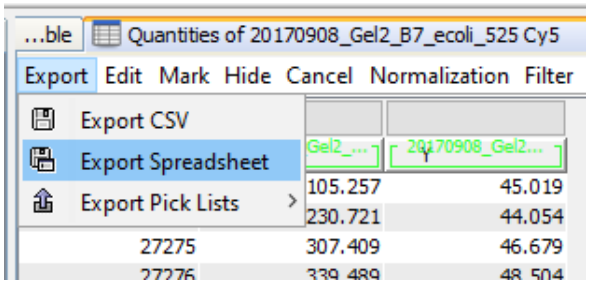


Figure 61: Export of data to Excel.

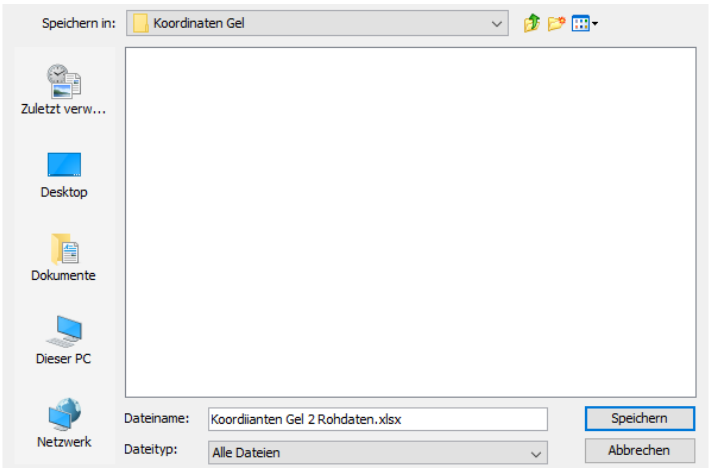
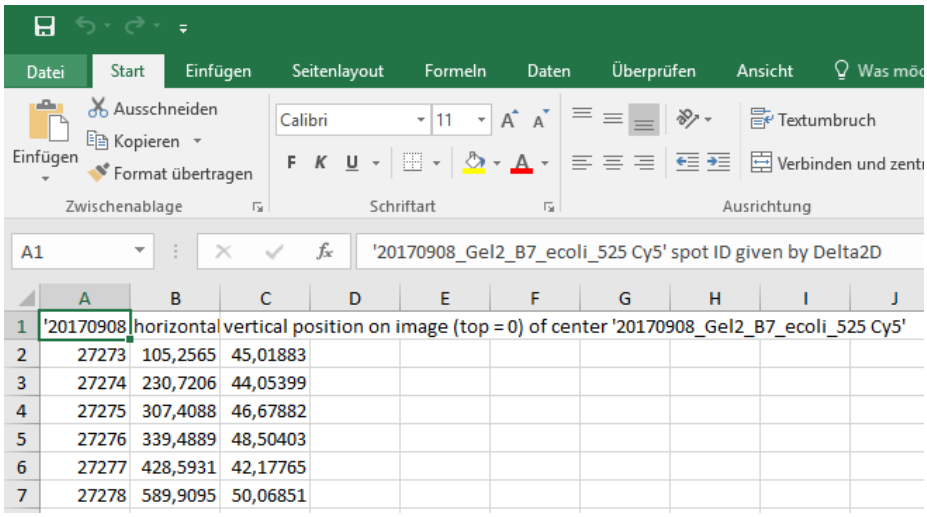


Figure 62: Saving coordinates as Excel file.



Datei Start Einfügen Seitenlayout Formeln Daten Überprüfen Ansicht

Ausschneiden Kopieren Format übertragen Einfügen Zwischenablage

Calibri 11 Schriftart

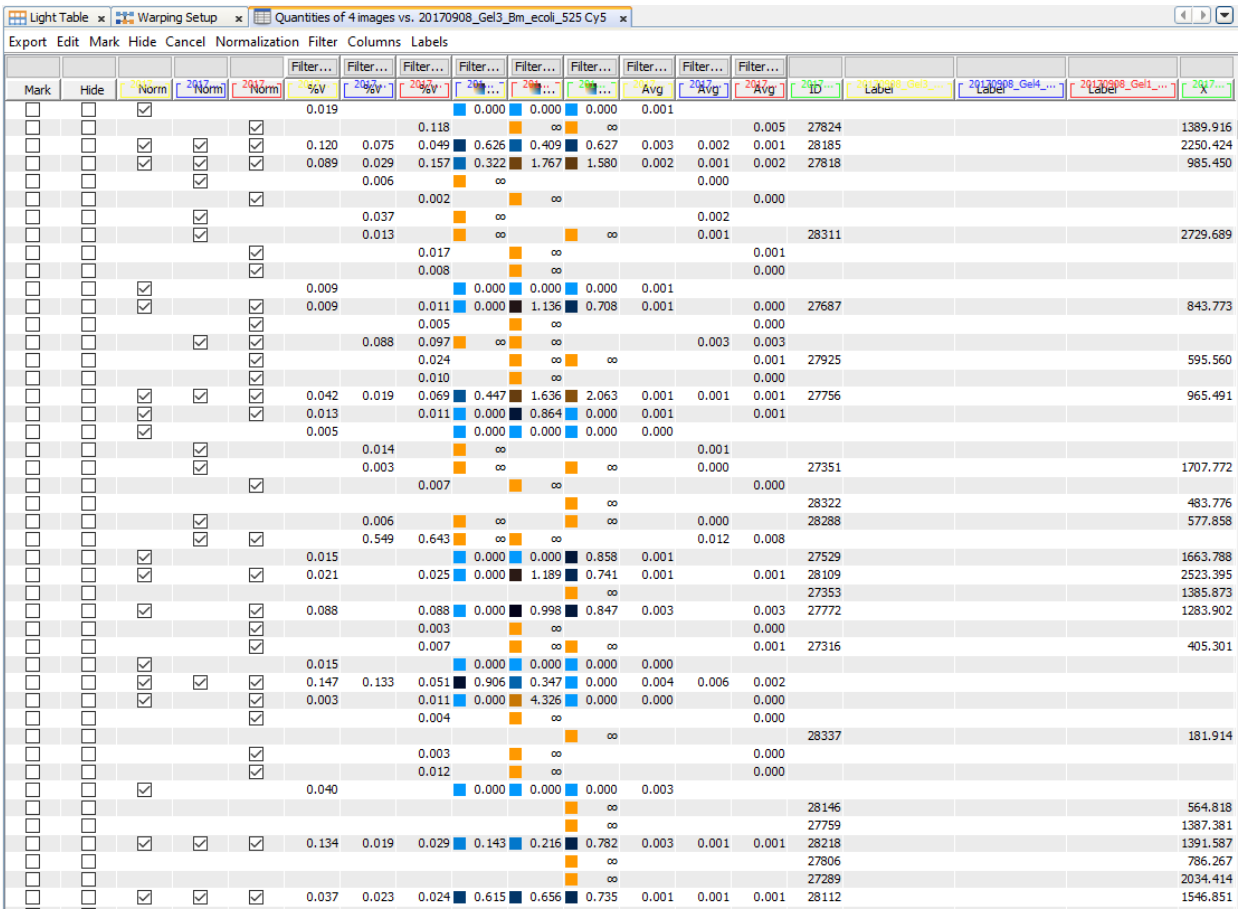
Textumbruch Verbinden und zentr Ausrichtung

A1 '20170908_Gel2_B7_ecoli_525 Cy5' spot ID given by Delta2D

	A	B	C	D	E	F	G	H	I	J
1	'20170908	horizontal	vertical position on image (top = 0) of center '20170908_Gel2_B7_ecoli_525 Cy5'							
2	27273	105,2565	45,01883							
3	27274	230,7206	44,05399							
4	27275	307,4088	46,67882							
5	27276	339,4889	48,50403							
6	27277	428,5931	42,17765							
7	27278	589,9095	50,06851							

Figure 63: Spot coordinates in Excel.

It is possible to export the coordinates of several gels at the same time. Spots on all gels must have been detected. All sample gels need to be selected while pressing the ‘Ctrl’ button and open ‘Quantitation Table’ (Figure 64).



Light Table | Warping Setup | Quantities of 4 images vs. 20170908_Gel3_Bm_ecoli_525 Cy5

Export Edit Mark Hide Cancel Normalization Filter Columns Labels

Mark	Hide	Norm	Norm	Norm	%V	%V	%V	Avg	Avg	Avg	ID	Label	X
☐	☐	☑			0.019		0.000 0.000	0.000	0.001				
☐	☐			☑		0.118	∞ ∞			0.005	27824		1389.916
☐	☐	☑	☑	☑	0.120	0.075	0.049 0.626 0.409	0.627	0.003	0.002	0.001	28185	2250.424
☐	☐	☑	☑	☑	0.089	0.029	0.157 0.322 1.767	1.580	0.002	0.001	0.002	27818	985.450
☐	☐		☑			0.006	∞			0.000			
☐	☐			☑		0.002	∞				0.000		
☐	☐		☑			0.037	∞			0.002			
☐	☐		☑			0.013	∞	∞		0.001	28311		2729.689
☐	☐			☑		0.017	∞				0.001		
☐	☐			☑		0.008	∞				0.000		
☐	☐	☑			0.009		0.000 0.000	0.000	0.001				
☐	☐	☑		☑	0.009		0.011 0.000 1.136	0.708	0.001		0.000	27687	843.773
☐	☐			☑		0.005	∞				0.000		
☐	☐		☑	☑		0.088	0.097 ∞ ∞			0.003	0.003		
☐	☐			☑		0.024	∞	∞			0.001	27925	595.560
☐	☐			☑		0.010	∞				0.000		
☐	☐	☑	☑	☑	0.042	0.019	0.069 0.447 1.636	2.063	0.001	0.001	0.001	27756	965.491
☐	☐	☑		☑	0.013		0.011 0.000 0.864	0.000	0.001		0.001		
☐	☐	☑			0.005		0.000 0.000	0.000	0.000				
☐	☐		☑			0.014	∞			0.001			
☐	☐		☑			0.003	∞	∞		0.000		27351	1707.772
☐	☐			☑		0.007	∞				0.000		
☐	☐						∞					28322	483.776
☐	☐		☑			0.006	∞	∞		0.000		28288	577.858
☐	☐		☑	☑		0.549	0.643 ∞ ∞			0.012	0.008		
☐	☐	☑			0.015		0.000 0.000	0.858	0.001			27529	1663.788
☐	☐	☑		☑	0.021		0.025 0.000 1.189	0.741	0.001		0.001	28109	2523.395
☐	☐						∞					27353	1385.873
☐	☐	☑		☑	0.088		0.088 0.000 0.998	0.847	0.003		0.003	27772	1283.902
☐	☐			☑		0.003	∞				0.000		
☐	☐			☑		0.007	∞	∞			0.001	27316	405.301
☐	☐	☑			0.015		0.000 0.000	0.000	0.000				
☐	☐	☑	☑	☑	0.147	0.133	0.051 0.906 0.347	0.000	0.004	0.006	0.002		
☐	☐	☑		☑	0.003		0.011 0.000 4.326	0.000	0.000		0.000		
☐	☐			☑		0.004	∞				0.000		
☐	☐						∞					28337	181.914
☐	☐			☑		0.003	∞				0.000		
☐	☐			☑		0.012	∞				0.000		
☐	☐	☑			0.040		0.000 0.000	0.000	0.003				
☐	☐						∞					28146	564.818
☐	☐						∞					27759	1387.381
☐	☐	☑	☑	☑	0.134	0.019	0.029 0.143 0.216	0.782	0.003	0.001	0.001	28218	1391.587
☐	☐						∞					27806	786.267
☐	☐						∞					27289	2034.414
☐	☐	☑	☑	☑	0.037	0.023	0.024 0.615 0.656	0.735	0.001	0.001	0.001	28112	1546.851

Figure 64: Extract ‘Quantitation Table’ for four gels.

6.2 Export of Coordinates of Warped Gels

The sample gel image in its warped form and FI-RG have to be open in ‘Dual View’. Coordinates can then be exported (Figure 65). The data file name for storage (Figure 66) describes to which gel image it was warped - in this example to FI-RG.

The exported image will be imported to the software as a new image and must be activated (Figure 67).

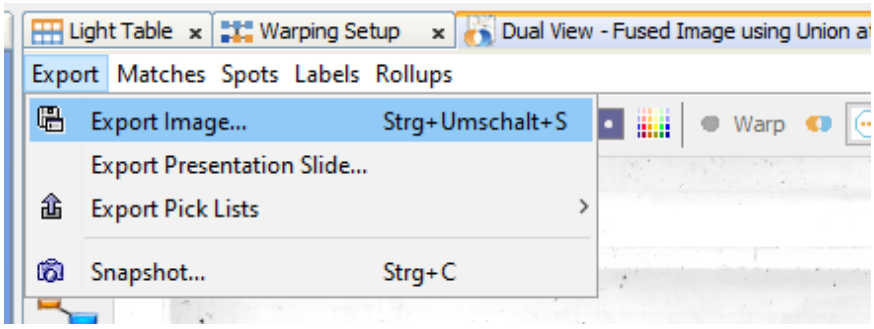


Figure 65: Export of warped gel images.

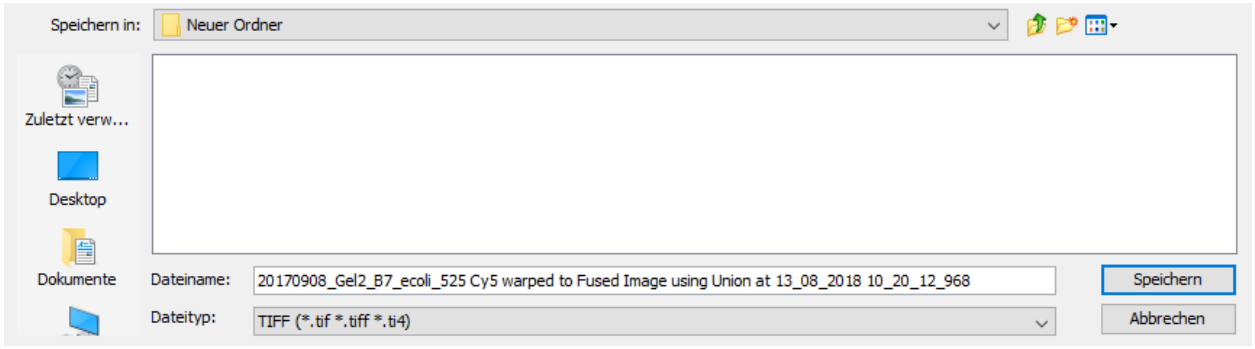


Figure 66: Saving warped images.

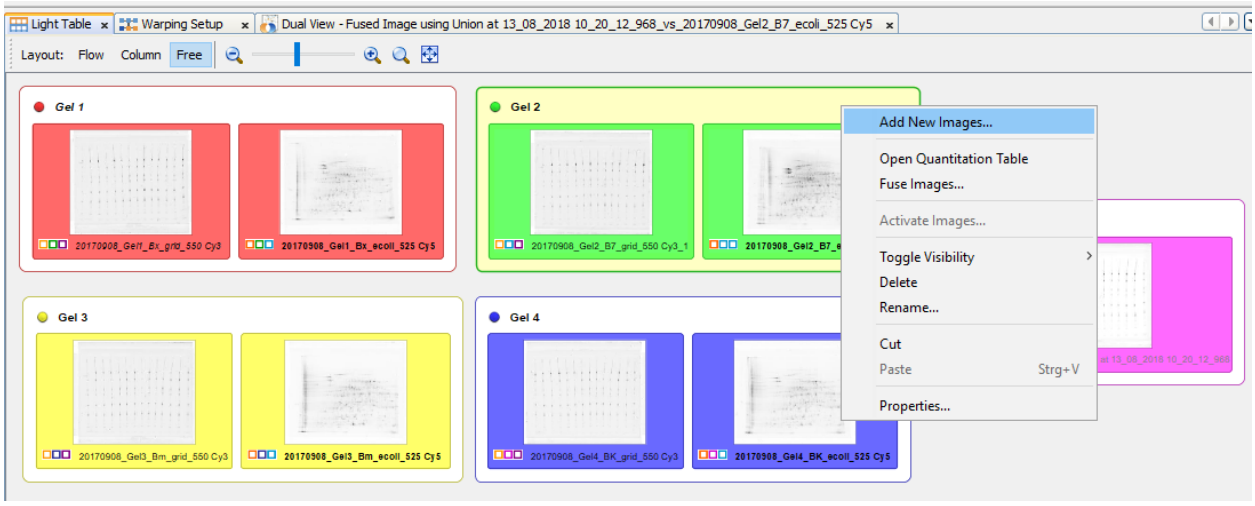


Figure 67: Import of warped gel image.

There are two possibilities for spot detection on the warped image. They can be detected as described above, or spots can be transferred from the original gel to the warped gel. The latter is the better option, if coordinates need to be compared. With spot transfer from one gel to another the spots have the same ID and are easier to find in the quantitation table.

Independently from these possibilities, the first step is to set the warp mode between the imported gel and FI-RG to ‘Identical’ (Figure 68).

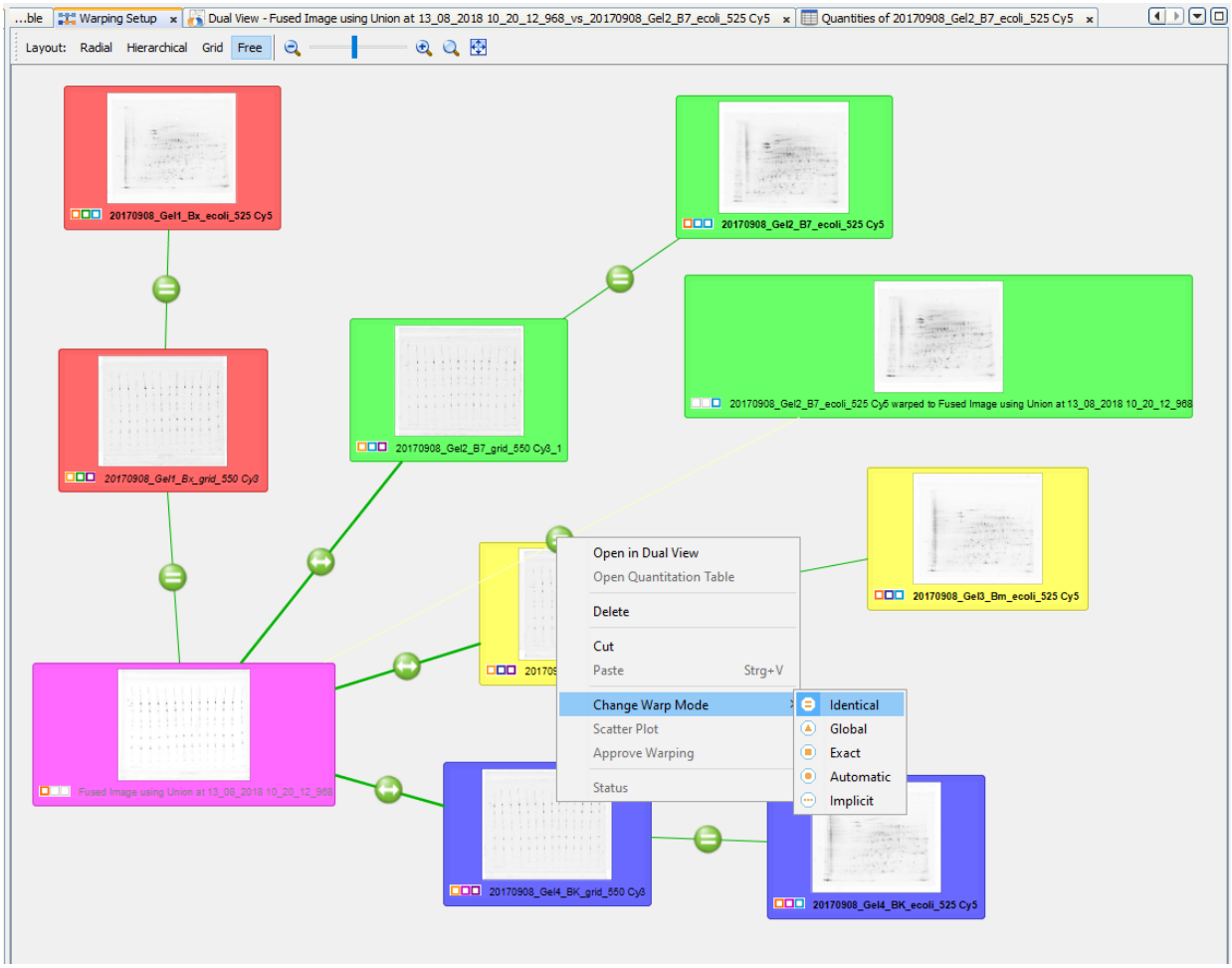


Figure 68: Warping relations between the warped gel image and FI-RG.

To transfer spots, ‘Project Explorer’ has to be opened. With the right mouse button the gel from which the spots shall be transferred has to be selected (Figure 69). Only spots within one group will be transferred. In the window shown in Figure 70 the gels can be selected to which spots will be transferred. Figure 71 shows the correct selection of gels. The coordinates on the warped image are then the same as on the original image (Figure 72).

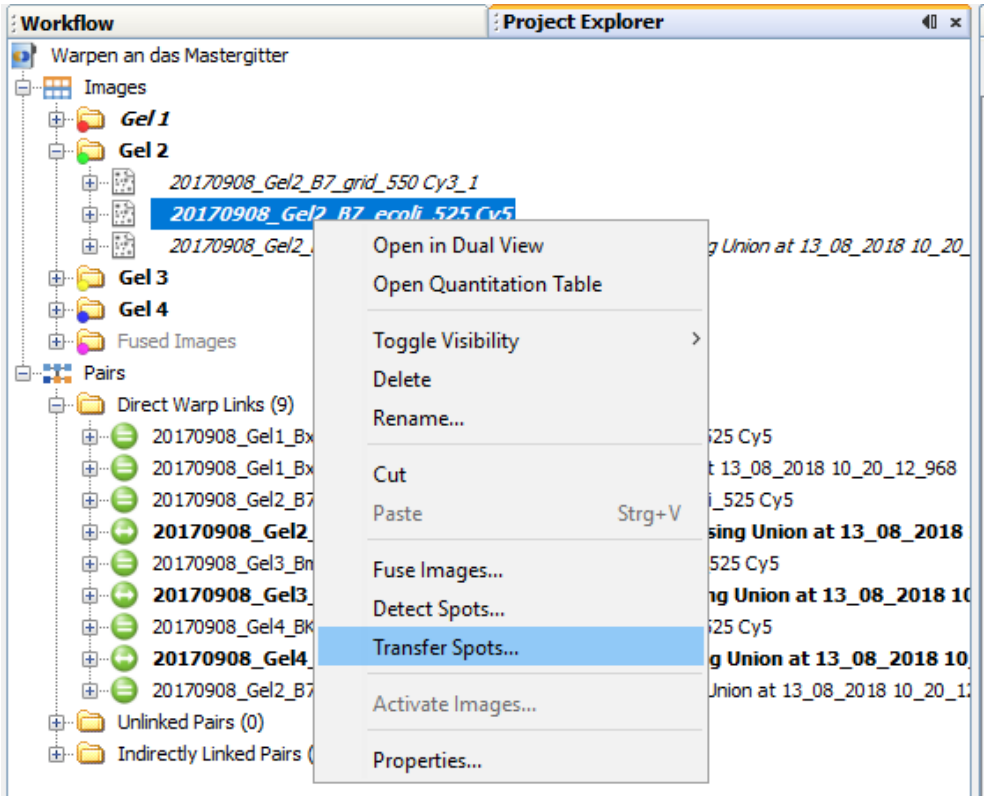


Figure 69:

'Project Explorer' – 'Transfer of Spots'.

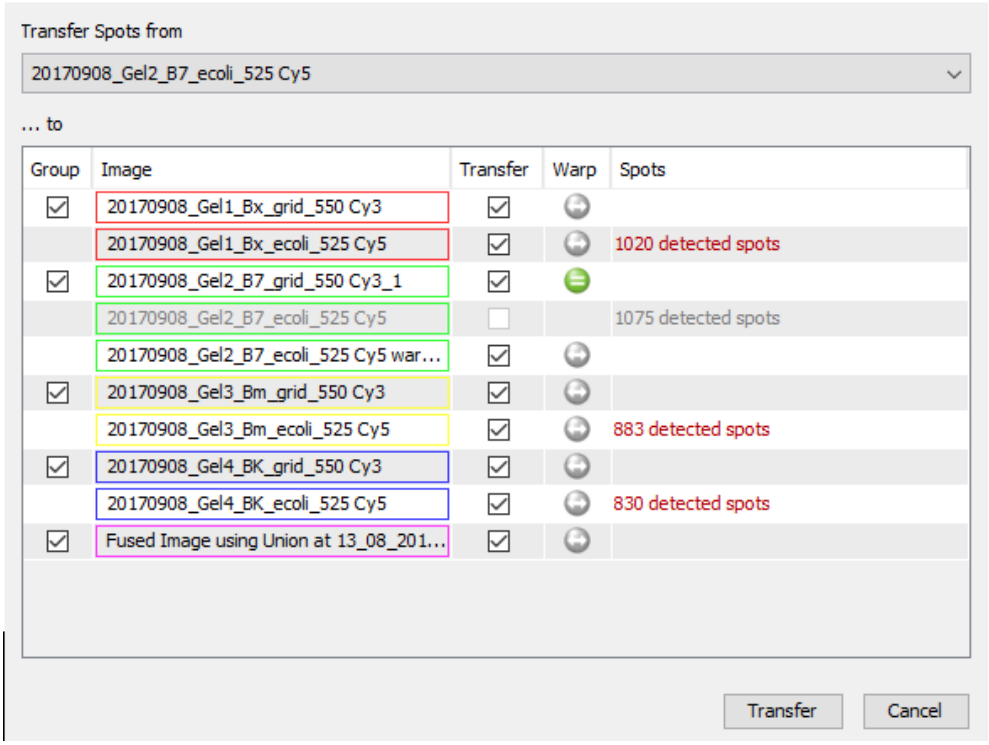


Figure 70:
'Transfer Spots from' before modification.

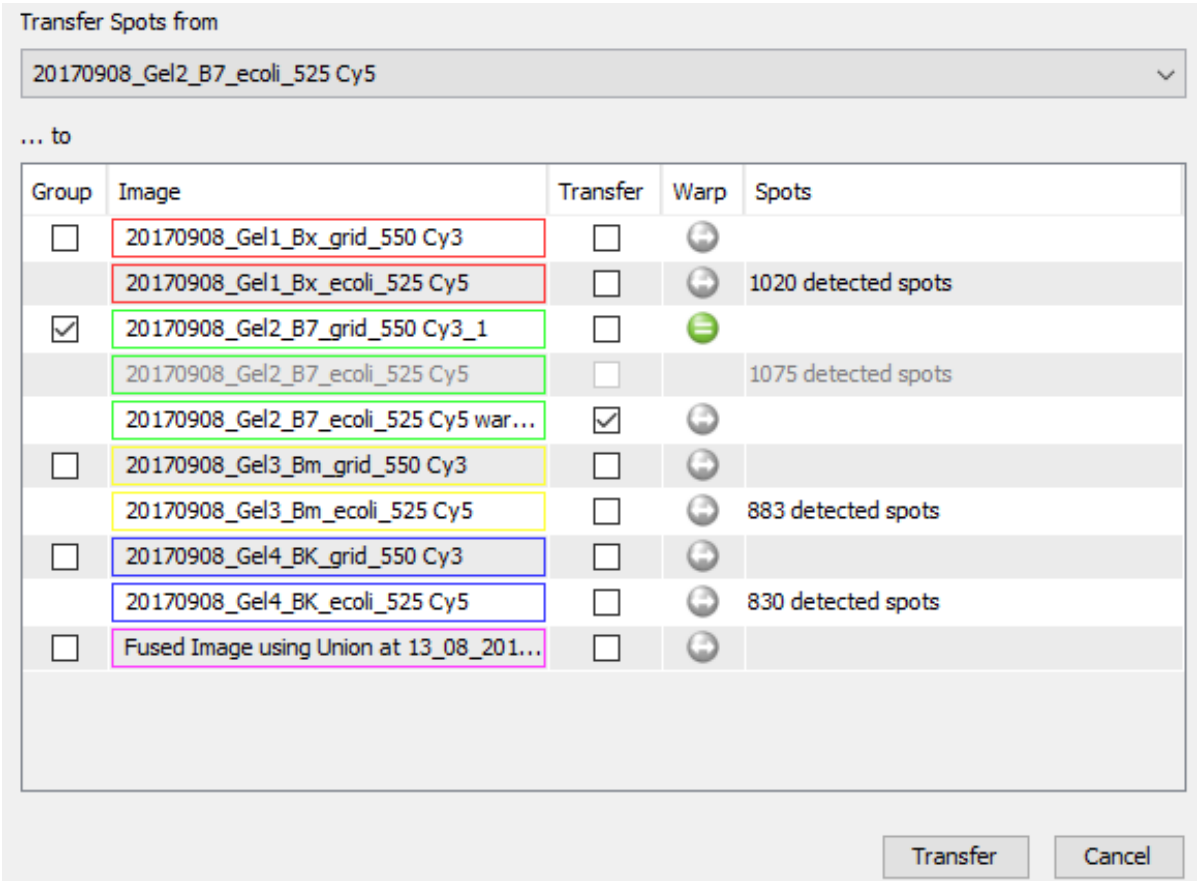


Figure 71: 'Transfer Spots from' after modification.

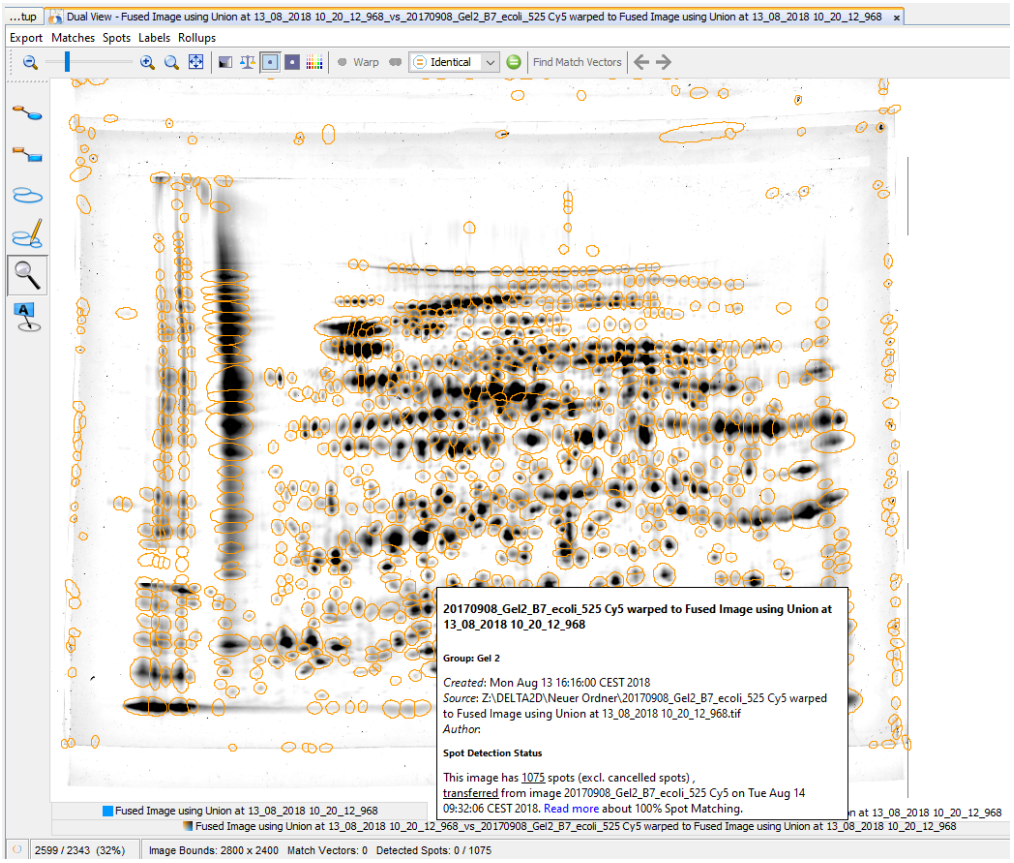


Figure 72: Transferred spots on warped gel image.

7. Referencing in Small Projects

It is possible to use one of the reference images form a dataset as master gel without creating a fusion gel as shown above. This reference grid should thus be of excellent quality. Import and activation, the assignment of groups and image attributes, as well as spot detection, transfer and export of coordinates were described before.

Grid gel images are warped to the master grid in the warp mode ‘Exact’. The corresponding sample gel images are set to ‘Identical’ to the grid gel image. The warped sample gel image is exported in its warped state and reimported. The warping setup with the warped sample gel image is shown in Figure 73.

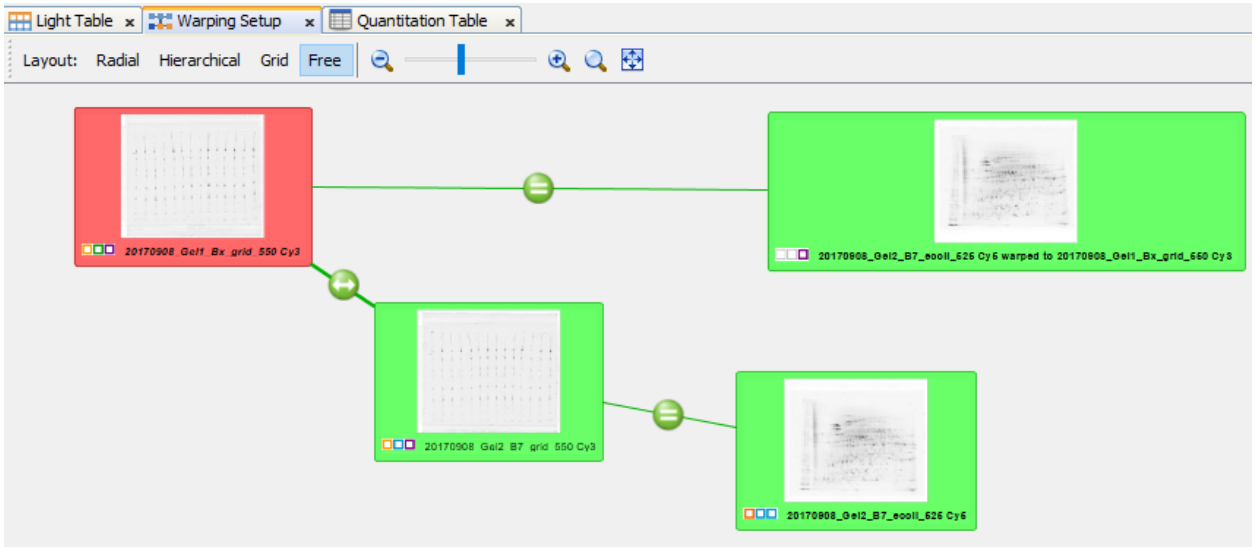


Figure 73: Warping setup with a master grid selected from the dataset.

8. Semi-quantitative Analysis

As the amount of standard protein used for referencing is known, the amount present for proteins of interest can be estimated. The normalized volume (%V) as well as the integrated grey volume without background (V) can be added to the quantitation table (Figure 74). Spots on the reference grid gel image must be have been detected. It is important to label each spot for better retrieval in the quantitation table (Figure 75). Sample spots need to be labeled as well for export of coordinates and volume to Excel.

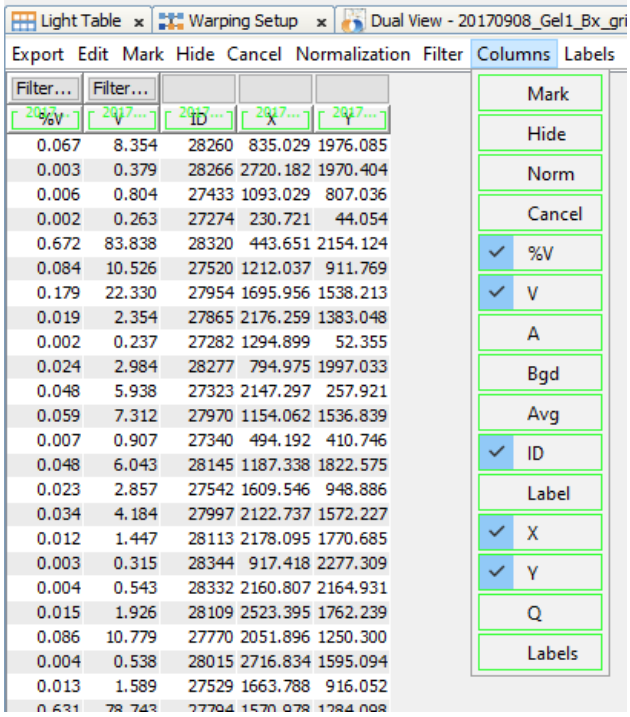


Figure 74: Volume in the quantitation table.

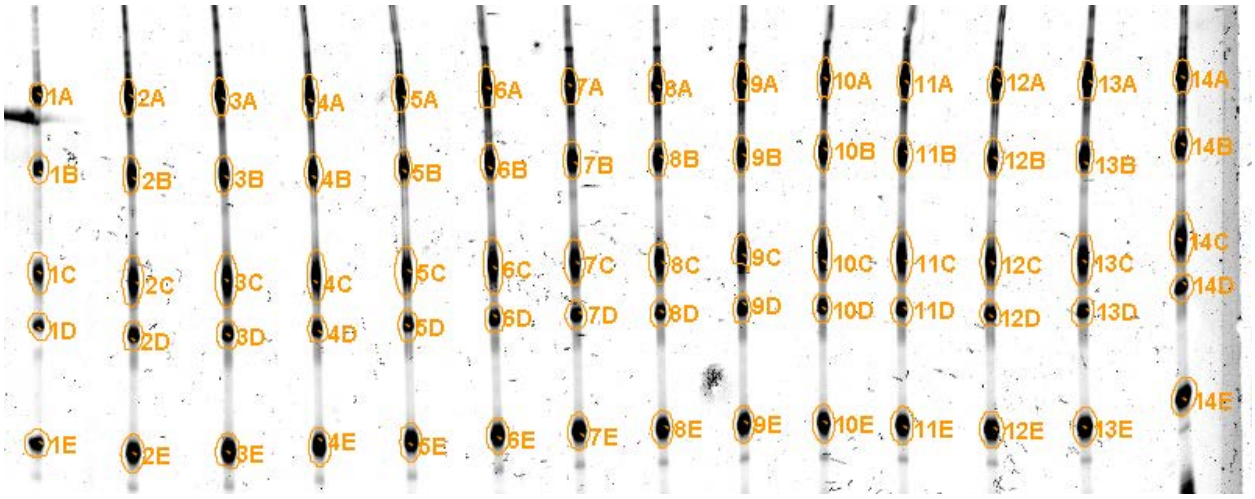


Figure 75: Labeled grid spots.

In order to find the grid spots closest to the analyte spot, 'Dual View' of both images is used (Figure 76).

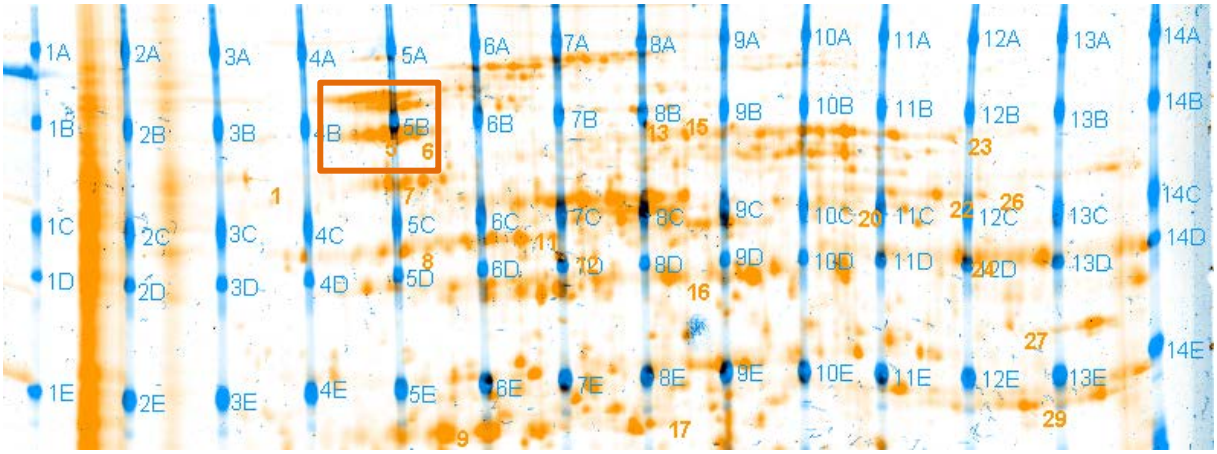
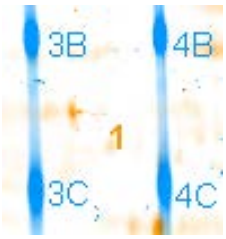


Figure 76: ‘Dual View’ of labeled spots.

An examplary calculation for sample spots 5 and 6 in reference to grid spot 5B of about the same molecular weight is shown in Table 1. As sample spot 6 has about the same intensity as grid spot 5B and spot 5 shows about a quarter of the intensity, the protein amounts result accordingly.

Tabel 1: Calculation of the amount of analyte protein present.

Spot	%V	Factor between grid and sample-Spot	m/Spot [ng]
5B	1,34	-	33,8
5	0,29	4,6	7,3
6	1,31	1,0	33,0



The references grid also serves as molecular weight marker. Using the y-coordinate, it can be calculated for spot 1 to 56 kDa (Figure 76, Table 2).

Tabel 2: Calculation of the molecular weight of analyte proteins.

Spot	**y**	**Mean y**	**Molecular weight [Da]**
1	976		56000
3B	872	870	69293
4B	869		
3C	1086	1081	42881
4C	1077		

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