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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.

<u>Note:</u> 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.

<u>Note:</u> 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.

<u>Note:</u> CoFGE relies on the use of the exact same lab procedure with respect to pl-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.

🖏 💸 🖏 🐻 💷 🙋
able x
Flow Column Free 🤤 🖳 🛑 💽 🔇
Projects - C:\Users\ackermad\.Delta2D\demopool X
Search: Clear 🕞 New 🗊 Delete
Name Type Author Date Description Demonstration Classic DECODON Feb 22, 2006 8: Delta 20 demonstration DEG Demonstration Classic DECODON Feb 22, 2006 8: Delta 20 demonstration
Dide Demonstrat Internal Standard DECODON Jun 25, 2011 4 Detta20 Dide d
Change Pool Open Cancel

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.

<u>Note:</u> 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.

Suchen in:	Dokume	nte	~	🏂 📂 🛄 •	
Zuletzt verw	Auswert Benutze Citavi 6	ung Mercator Gele rdefinierte Office-Vorlagen Ordner			
Desktop Dokumente Dieser PC					
Netzwerk	Dateiname: Dateityp:	Auswertung Mercator Gele All Directories			~
				OK	Cancel

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

?	There is no valid pool in the path "C:\Users\a_nipp01\Documents\Auswertung Mercator Gele". Do you really want to create a new pool in this directory ?				
		Ja	Nein		

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 the window shown in

Figure 6 appears in which project properties can be defined. It is possible to change these properties later.

gellmagePairs	08.08.2018 10:15	Dateiordner
📙 gellmages	08.08.2018 10:15	Dateiordner
nojects	08.08.2018 10:15	Dateiordner
📙 thumbnails	02.08.2018 15:38	Dateiordner

Figure 4: Pool data structure.

Search:		Clear	📑 New	💼 Delete
Name	Туре	Author	Date	Description
		Change Pool	Open	Cancel

Figure 5: Project overview.

Project Name	Project 1
Author	
Date	August 9, 2018 9:18 AM
Туре	Classic ~
Statistics over	Normalized Volume \checkmark
Description	
	Change Pool OK Cancel

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.

Search:		Clear	🕵 New	<u> Delete</u>
Name	Туре	Author	Date	Description
Erstellung eines	. Classic	Alina Nippes	Aug 9, 2018 11:	Veranschaulichu
		Change Pool	Open	Cancel

Figure 7: Project in pool overview.

Project Edit Images Pool R	eports Window Tools Help		
136,7/922,0MB 🗳 🚺	💽 🗎 🗶 🔲 🛍 😻 🕘 • 🥑 📥 2	🖲 💽 🖥	n 🕞 🖓 🐝 🖏 🛃 🛄 🙋
Workflow	💷 × 🔅 Project Explorer		🔛 Light Table 🗙 👯 Warping Setup 🗴 🖽 Quantitation Table 🗴
Project: Erstellung ei	nes Master/Theoretischen Gitters		Layout: Flow Column Free 🔍 — 🔍 🔍 🔯
Setup Project		8	
Erstellung eines Mas	ster/Theoretischen Gitters		
Import your images into this	project.		
Open the Light Table to orga	anize images in replicate groups.		Group 1
Groups (add a group)			
• Group 1 (no images, add	l)		
• Group 2 (no images, add	l)		
Use the Image Attributes dia	log to assign samples, channels, and gels for your images.		
2 Warp Images		8	
Oetect and Quantify	Spots	۲	
4 Analyze Expression P	rofiles	\otimes	
6 Present Results		8	
			Group 2
Image Regions 🔗 Job Ma	nager		

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.

<u>Note:</u> 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.

Steps	ielect images	
Select images	Suchen in: 📙 20170908_Gel1_Bx_550-525.dir 🗸 🕫 💬 🖽 🗸	
	Image: Substrain of Substraint State 20170908_Gel1_Bx_ecoli_525 Cy5 - Kopie.tif Image: Substraint State 20170908_Gel1_Bx_grid_550 Cy3 - Kopie.tif Image: Substraint State 20170908_Gel1_Bx_grid_550 Cy3 - Kopie.tif Image: Substraint State 20170908_Gel1_Bx_grid_550 Cy3 - Kopie.tif Image: Substraint State 20170908_Gel1_Bx_grid_550 Cy3.gel	
	Desktop	
	Dokumente	
	Dieser PC	
	Netzwerk	
	Dateiname: Dateityp: Supported Image Formats (*.tif *.tiff *.ti4 *.jpg *.jpeg *.png *.pnm *.gif *.bmp *.gel *.mel *.inf) v	
	< Back Next > Finish Cance	el l

Figure 9: Import gel images, 1st window.

Steps	20170908_Gel1_Bx_grid_550 Cy3.gel		
 Select images 20170908 Gel1 Bx grid 	General	Image Preparation	
550 Cy3.gel	Name 20170908_Gel1_Bx_grid_550 Cy3		\$ ₼ 🖬
	Date August 14, 2018 10:29 AM	Original	Preview
	Author		
	Image		
	Source File X:\Gele\Mercator\2017\20170908_4er E		
	Calibration Typhoon9400 1_21025 V	· · · · · · · · · · · · · · · · · · ·	
	Format image/tiff		
	Subformat		
	Grouping		
	Sample Not assigned 🗸	1.1 4	C. F. San Contraction
	Gel Not assigned V	1 5 1 1	
	Channel Cy3 V		
	Description		
			1
		Remove black 0 🔷 or white 0 🛓	speckles.
		< Back	Next > Finish Cancel

Figure 10: Import gel images, 2nd window.

ginal	Preview	using

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'.

Select Images to Activate:				
Group	Image	Activate	Select All	Figure 12: Gel image activation.
	20170908_Gel1_Bx_grid_5		Select None	
User Nar	User Name:			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
Passwor	d:		a group and choosing 'Rename'.	
		Activate	Cancel	

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.

Name	Group 3	
Color		~
	OK	Cancel

it: Flow Column Free 🔍 —			
Gel 1	Gel 2	Gel 3	• Gel 4
20170908_Gel1_Bx_grid_550 Cy3	20170908_Gel2_B7_grid_550 Cy8_1	20170908_Gel3_Bm_grid_550 Cy8	20170908_Gel4_BK_grid_550

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.

Delta2D 4.8.0 - Erstellung eines Master/Theoretischen Gitters (C:\Users\a_nipp01\Documents\Auswe Project Edit Images Pool Reports Window Tools Help





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.

Sample Gel Channel					
Project	Gel	Sample	Channel	.	
 Distributer great 20170908_Gel1_Bx_grid_550 Cy3 20170908_Gel2_B7_grid_550 Cy3_1 20170908_Gel3_Bm_grid_550 Cy3 20170908_Gel4_BK_grid_550 Cy3 Sample A Sample B 	Not assig Not assig Not assig Not assig	Not assig Not assig Not assig Not assig	□ Cy3 □ Cy3 □ Cy3 □ Cy3 □ Cy3		Figure 16: 'Image Attributes' – 'Sample', before assignment.
Sample C Sample D Sample E Sample F Sample F					
Sample H					
		OK		Cancel	

Sample Gel Channel			
Project	Gel	Sample	Channel
Erstellung Mastergitter			
Sample A			
🚊 🖳 Sample B			
20170908_Gel1_Bx_grid_550 Cy3	Not assig	🗖 Sample B	Cy3
20170908_Gel2_B7_grid_550 Cy3_1	Not assig	🗖 Sample B	Cy3
20170908_Gel3_Bm_grid_550 Cy3	Not assig	🗖 Sample B	Cy3
20170908_Gel4_BK_grid_550 Cy3	Not assig	🗖 Sample B	Cy3
Sample C			
Sample D			

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.

Sample Gel Channel				
Project	Gel	Sample	Channel	₿.
Erstellung Mastergitter				
20170908_Gel1_Bx_grid_550 Cy3	Not assig	🗖 Sample B	Cy3	
20170908_Gel2_B7_grid_550 Cy3_1	Not assig	🗖 Sample B	Cy3	
20170908_Gel3_Bm_grid_550 Cy3	Not assig	🗖 Sample B	Cy3	
20170908_Gel4_BK_grid_550 Cy3	Not assig	🗖 Sample B	Cy3	
Gel I				
Gel IV				
Gel VI				
		OK	Car	ncel

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

Sample Gel Channel				
Project	Gel	Sample	Channel	
Erstellung Mastergitter				1
Gel I Gel I 20170908_Gel1_Bx_grid_550 Cy3	🗖 Gel I	Sample B	Суз	
Gel II 20170908_Gel2_B7_grid_550 Cy3_1	🗖 Gel II	Sample B	Суз	
Gel III	🗖 Gel III	Sample B	Суз	
Gel IV	Gel IV	Sample B	Cy3	
Gel V		_		
Gel VI				

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.

Sample Gel Channel			
Project	Gel	Sample	Channel
35S met pulse			
- Amido Black			
Colloidal Coomassie			
Coomassie			
Cy2			
Ė.⊶ ⊡ Cy3			
20170908_Gel1_Bx_grid_550 Cy3	🗖 Gel I	🗖 Sample B	🗖 Cy3
20170908_Gel2_B7_grid_550 Cy3_1	🗖 Gel II	🗆 Sample B	🗖 Cy3
	🗖 Gel III	🗖 Sample B	🗖 Cy3
20170908_Gel4_BK_grid_550 Cy3	🗖 Gel IV	Sample B	🗖 Cy3
Cy5			
Diamond Pro Q			
Emerald Pro Q			

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.

<u>W</u> ind	low <u>T</u> ools <u>H</u> elp	
	Workflow	Strg+Umschalt+0
,□	Project Explorer	Strg+Umschalt+1
	Light Table	Strg+Umschalt+2
3	Warping Setup	Strg+Umschalt+3
ö	Dual View	Strg+Umschalt+4
	Quantitation Table	Strg+Umschalt+5
Ħ	Image Regions	Strg+Umschalt+6
d,	Expression Profiles	Strg+Umschalt+7
	Color Coding	Strg+Umschalt+8
¢	Job Manager	Strg+Umschalt+9
э.	Analysis	
	Project Matrix	
6	P <u>r</u> operties	
	<u>Full Screen</u>	Alt+Umschalt+Eingabe
	Close Window	
	Maximize Window	
	<u>U</u> ndock Window	Alt+Umschalt+D
	Close <u>A</u> ll Documents	
	Close Other Documents	
	<u>D</u> ocuments	
	Reset <u>W</u> indows	

Figure 21: Start 'Warping-Setup'.

🔠 Light Table 🗙 📰 Warping Setup 🗙			
Layout: Flow Column Free 🔍 —			
:	• • • • • • • • • • • • • • • • • • •		
Gel 1	Gel 2	🥥 Gel 3	• Gel 4
20170908_Gel1_Bx_grid_550 Cy3	20170908_Gel2_B7_grid_550 Cy3_1	20170908_Gel3_Bm_grid_550 Cy8	20170908_Gel4_BK_grid_550 Cy3

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 ³.



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.

Export Mate	hes Spots Label	s Rollups						
Q —		۹ 🔁 🔊	- <u>1</u>	💛 Warp	Automatic	~ 📀 F	ind Match Vectors 🖌 🔶	7
		بمستعبر رابر بسراب	ere missionante entrat		موجدها بدر آن الجمار ال		Smart Vectors	-
	11.						Find new match vectors automatically, using Smart Vectors Technology.	-
H	T.							-

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.

Review direct warp Double click on any pai	ings r in the list to open it in th	e Dua	View.		Del
1st Image 20170908 Gel1 Bx	2nd Image 20170908 Gel2 B7	6	Status Review	Approve Approve	DECI Figure 27: N
20170908_Gel1_Bx	20170908_Gel3_Bm	0	Review	Click to a	approve all matchvectors 'Approved'.
20170908_Gel1_Bx	20170908_Gel4_BK	٢	Review	Approve	Del

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.



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.

Master Ir	nage	20170908_Gel1_Bx_grid_550 Cy3 ~				~	Description
Apply Ca	libration						Select Master Image
Process I	mages Before Fusion	n Remove Background				~	Select the master image for fusion. All selected
		Amplitude Res	cale			~	images will be warped to the selected master image before the fusion starts.
Fusion Ty	/pe	Union				~	Note: If there are images for which no valid warning
Adjust In	nage Sizes	Common Regi	on			~	to the selected fusion master exists, the fusion
Apply Inv	verse Calibration						function will not be available.
Process F	Fused Images	Amplitude Dec	cale				
FIOCESSI	useu images		cale			~	
T T	- F	Don't Hilter				~	
Group	Image		Fuse	Warp	Calibration		
	20170908_Gel1_Bx_0	grid_550 Cy3			Typhoon9400 1_21025		
	20170908_Gel2_B7_0	grid_550 Cy3_1		٢	Typhoon9400 1_21025		
	20170908_Gel3_Bm_	grid_550 Cy3	\checkmark	٢	Typhoon9400 1_21025		
	20170908_Gel4_BK_c	grid_550 Cy3	\checkmark	٢	Typhoon9400 1_21025		
							Fuse Cancel

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.



Activate Images The fused image is not activated yet.

Please activate fusion images, its free. 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.

Select In	nages to Activate:										
Group	Image	Activate	Cost	Comment	Select All						
\checkmark	Fused Image using Union at	\checkmark	0	Free fusion image	Select None						
						Fig	ure 36: Ad	ctivatio	on of	fused	image.
							Activation I You have activated	Report			108 credits
							submitted	items	free	paid	credits
							Gel Image	1	1	0	0
Liser Nar	ne'						total	1	1	0	0
							charge:				0 credits
	1.						current balance:				108 credits
Passwor	a:				Activate Cancel		For more details plea	se review the c	confirmation OK	email or yo	ur consumable accor



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.



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.

Sample Gel Channel				
Project	Cel	Sample	Channel	.
Project E Coli 20180827	001	Sample	Chariner	-
i → □ Sample A				-
20170908_Gel1_Bx_ecoli_525 Cy5	Not assig	Sample A	Cy5	
20170908_Gel2_B7_ecoli_525 Cy5	Not assig	Sample A	Cy5	
20170908_Gel3_Bm_ecoli_525 Cy5	Not assig	Sample A	Cy5	
Sample B	INOU dosig	LI Sample A		
20170908_Gel1_Bx_grid_550 Cy3	🗖 Gel I	Sample B	Су3	
20170908_Gel2_B7_grid_550 Cy3	🗖 Gel II	Sample B	Cy3	
20170908_Gel3_Bm_grid_550 Cy3	🗖 Gel III	Sample B	Cy3	
20170908_Gel4_BK_grid_550 Cy3	Gel IV	Sample B	Cy3	
Fused Image using Union at 27_08_20.	I NOT assig	Sample b	Not assig	

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

Sample Gel Channel			
Project	Gel	Sample	Channel
Warpen an das Mastergitter			
	2 Not assigned	Sample A	Not assigned
🖨 🗖 Gel I			
20170908_Gel1_Bx_ecoli_525 Cy5	🗖 Gel I	Sample A	Cy5
20170908_Gel1_Bx_grid_550 Cy3	🗖 Gel I	Sample B	Cy3
🚊 🗖 Gel II			
20170908_Gel2_B7_ecoli_525 Cy5	🗖 Gel II	Sample A	Cy5
20170908_Gel2_B7_grid_550 Cy3_1	🗖 Gel II	Sample B	Cy3
🖨 🗖 Gel III			
20170908_Gel3_Bm_ecoli_525 Cy5	Gel III	Sample A	Cy5
20170908_Gel3_Bm_grid_550 Cy3	Gel III	Sample B	Cy3
🖨 🗖 Gel IV			
20170908_Gel4_BK_ecoli_525 Cy5	Gel IV	Sample A	Cy5
20170908_Gel4_BK_grid_550 Cy3	Gel IV	🗖 Sample B	Cy3
Gel V			
Gel VI			

Figure 41: 'Image Attributes' –'Gel' for sample gels and fused image.

Sample Gel Channel					
Project	Gel	Sample	Channel		
				^	
Cy3 	Gel I Gel II Gel III Gel IV	Sample B Sample B Sample B Sample B	Cy3 Cy3 Cy3 Cy3 Cy3	ł	
 Cy5 20170908_Gel1_Bx_ecoli_525 Cy5 20170908_Gel2_B7_ecoli_525 Cy5 20170908_Gel3_Bm_ecoli_525 Cy5 20170908_Gel4_BK_ecoli_525 Cy5 Diamond Pro Q 	Gel I Gel II Gel III Gel III	Sample A Sample A Sample A Sample A	Cy5 Cy5 Cy5 Cy5 Cy5		
 20170908_Gel3_Bm_grid_550 Cy3 20170908_Gel4_BK_grid_550 Cy3 Cy5 20170908_Gel1_Bx_ecoli_525 Cy5 20170908_Gel3_Bm_ecoli_525 Cy5 20170908_Gel3_Bm_ecoli_525 Cy5 20170908_Gel4_BK_ecoli_525 Cy5 Diamond Pro Q Emerald Pro Q 	Gel III Gel IV Gel I Gel II Gel III Gel IV	Sample B Sample B Sample A Sample A Sample A Sample A	Cy3 Cy5 Cy5 Cy5 Cy5 Cy5 Cy5		

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 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.



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.

0	/	$\boldsymbol{\alpha}$	_	
4	Open in Dual View			
	Open Quantitation Tabl	e		
	<u>D</u> elete			
	Cu <u>t</u>			
	<u>P</u> aste	Strg+V	-6	
	Change Warp Mode	;	•	<u>I</u> dentical
	Scatter Plot		۲	<u>G</u> lobal
r uisin	Approve Warping			<u>E</u> xact
	Status		•	<u>A</u> utomatic
	56665		\odot	l <u>m</u> plicit

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.

Eused Image using Union at 13_08_2018 10_20_12_968 📒 20170908_Gel2_B7_ecol_525 Cy5 📲 Fused Image using Union at 13_08_2018 10_20_12_968_vs_20170908_Gel2_B





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

Fused Image using Union at 13_08_2018 10_20_12_968 20170908_Gel2_87_ecol_525 Cy5 Fused Image using Union at 13_08_2018 10_20_12_968_vs_20170908_Gel2_8



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.

🔠 Ligh	t Table 🗴	T V	Varping Setup 🛛 🗙 💦 Dual View - Fused Image using Union at 13_08_2018 10_20_12_9	968_vs_20170908_Gel2_B7_ecoli_525 Cy5 🗙
Export	Matches	Spot	s Labels Rollups	
9		Λ	Detect Spots on 20170908_Gel2_B7_ecoli_525 Cy5	Match Vectors \leftarrow \rightarrow
		Л	Detect Spots on Fused Image using Union at 13_08_2018 10_20_12_968	
~		Ť.	Delete	
		6	Import	>
~		8	Export	>
0			Show Table Strg+T	and the state of the
			Show Scatterplot Strg+L	1월 27년 소란 관리 나라?
			Show Hidden Spots	
			Show Canceled Spots	
	j.		Background Region	>
Å			I Contraction of the second se	1

Figure 54: Spot detection toolbar.



Figure 55: Detected spots on sample gel 2.



Figure 56: 'Spot Selection Tool'.



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

× X Warping Setup × Open Quantitation Table since (Figure 58).

ble	🔢 Quantitie	s of 2017	70908_Gel2_	B7_ecoli_5	25 Cy5	×	💶 Warping Se	tup	× 👸 Dual Viev	^
Export	Edit Mark	Hide	Cancel No	rmalizatio	n Filter	Co	lumns Labels			
			Filter	Filter			Filter		Filter	
C ²⁰ Mark] [²⁹ Hide]	²⁰ Norm	[²⁰ %√·]	²⁰ Åvg [.]]	- 201702P8	3_Gel	2] 2007	[²⁰	170908_Gel2]	C
		\checkmark	0.848	0.035			0.839)		
		\checkmark	0.143	0.013			0.383	3		
		\checkmark	0.006	0.001			0.140)		
		\checkmark	0.002	0.000			0.162	2		
		\checkmark	0.045	0.004			0.195	5		
		\checkmark	0.021	0.005			0.258	3		
		\checkmark	0.004	0.000			0.129)		
		\checkmark	0.016	0.002			0.183	3		
		\checkmark	0.170	0.006			0.263	3		
		\checkmark	0.003	0.001			0.174	ŧ		
		\checkmark	0.031	0.004			0.24	L		
		\checkmark	0.013	0.002			0.186	5		
		\checkmark	0.019	0.002			0.180)		
		\checkmark	0.019	0.002			0.173	3		
		\checkmark	0.113	0.006			0.329)		
		\checkmark	0.019	0.004			0.220)		
		\checkmark	0.096	0.004			0.264	1		
		\checkmark	0.111	0.003			0.369)		
		\checkmark	0.071	0.009			0.217	7		

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).

ble	🛛 Quantitie	es of 20170)908_Gel2_	_B7_ecoli_52	5 Cy5	×	🕻 Warping	Setup
Export	Edit Mark	Hide C	ancel No	ormalization	Filter	Colu	mns Lab	els
			Filter	Filter		~	Mark	
²⁰ Mark	²⁰¹⁷ Hide	²⁰ Norm	²⁰ ₩	²⁰ Avg ⁻]	20170208	1	Llide	
		\checkmark	0.848	0.035		×	Hide	Э
		\checkmark	0.143	0.013		\checkmark	Norm	3
		\checkmark	0.006	0.001				þ
		\checkmark	0.002	0.000			Cancel	2
		\checkmark	0.045	0.004		\checkmark	%V	5
		\checkmark	0.021	0.005				В
		\checkmark	0.004	0.000			V	Э
		\checkmark	0.016	0.002				3
		\checkmark	0.170	0.006			А	3
		\checkmark	0.003	0.001			Bad	4
		\checkmark	0.031	0.004			- 5	1
		\checkmark	0.013	0.002		\sim	Avg	5
		\checkmark	0.019	0.002			ID	p
		\checkmark	0.019	0.002				3
		\checkmark	0.113	0.006		\checkmark	Label	Э
		\checkmark	0.019	0.004				Þ
		\checkmark	0.096	0.004			Х	4
		\checkmark	0.111	0.003			Y	Э
		\checkmark	0.071	0.009				7
		\checkmark	0.068	0.003		\checkmark	Q	Э
		\checkmark	0.028	0.004		1	Labals	1
		\checkmark	0.044	0.007		~	Lapels	2
		\checkmark	0.763	0.026			0.5	559

Figure 59: Selection of columns in the quantitation table.

ble 🔲 Q	uantities	of 201	170908_0	el2_B7_	ecoli_525	Cy5	×
Export Edit	Mark	Hide	Cancel	Norma	lization	Filter	Colu
39170908_Ge	12] [201709	08_Gel2	·] [294	70908_Ge	2]	
2	27273		105.2	57	49	5.019	
2	27274		230.7	21	44	4.054	
2	27275		307.4	09	46	5.679	
2	27276		339.4	39	48	3.504	
2	27277		428.5	93	42	2.178	
2	27278		589.9	10	50	0.069	
2	27279		737.8	13	50). 135	
2	27280		176.4	94	64	1.843	
2	27281		1245.1	81	5	1.909	
2	27282		1294.8	99	52	2.355	

Figure 60: Extract quantitation table with coordinates and ID

ble		🗌 Qu	antities	of 201	170)908_G	Gel2_B7_ecoli_525 Cy5	
Expo	ort	Edit	Mark	Hide	С	ancel	Normalization Filte	r
8	Ex	port	CSV					
6	Ex	port	Spread	sheet		Gel2] [20170908_Gel2]	
命	Ex	port	Pick Li	sts	>	105.2	257 45.019	
		2010				230.7	721 44.054	
		2	7275			307.4	109 46.679	
		2	7276			339.4	48 9 48 504	

Figure 61: Export of data to Excel.

Speichern in:	Koordina	aten Gel	~ 🎾	• 🖽 🏷
Zuletzt verw				
Desktop				
Dokumente				
Dieser PC				
) Netzwerk	Dateiname: Dateityp:	Koordiianten Gel 2 Rohdaten.xlsx Alle Dateien	~	Speichern Abbrechen

Figure 62: Saving coordinates as Excel file.

	. 5-1													
D	atei St	art Einfi	igen	Seit	enlay	out	Formel	n Dat	en	Überprü	fen	Ansicht	Q	Was mö
		usschneiden		Calib	ri		- 11	ĂĂ	=	$\equiv \equiv$	87 -	루 Tex	tumb	ruch
Ein	fügen	ormat übertr	agen	F	к <u></u>	-	🖽 - 🕹	• <u>A</u> •	≡	$\equiv \equiv $	€∃→∃	🖶 Ver	binde	n und zent
	Zwische	nablage	Es.			Sch	riftart	G.			A	usrichtun	g	
A	L	• : :	× .	· .	f _x	'20	170908_G	el2_B7_e	coli_	525 Cy5'	spot ID	given b	y Del	ta2D
	А	в	с		D)	E	F		G	н		i i	J
1	20170908	horizonta	vertic	al pos	sitior	n on	image (to	p = 0) of (ente	er '201709	08_Gel	2_B7_eo	oli_5	525 Cy5'
2	27273	105,2565	45,01	883										
3	27274	230,7206	44,05	399										
4	27275	307,4088	46,67	7882										
5	27276	339,4889	48,50	403										
6	27277	428,5931	42,17	765										
7	27278	589,9095	50,06	5851										

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 ×	🚺 Warp	ing Setup	× 🔲 Qu	antities of	f 4 images	vs. 20170	908_Gel3_	Bm_ecoli	525 Cy5	×							
Export	Edit Mark	Hide	Cancel No	rmalizatio	on Filter	Column	s Labels											
					Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter	Filter					
Mark	Hide	²⁰ Norm	1 ²⁹ 10rm]	²⁹ Norm]	²⁰ %V-1	²⁰ ₩']	[²⁰ ₩']	201 :: T	[²	[² ∰∷.⊺	²⁰ Ávg]	[² %]7g;]	²⁰ A7g·]	[²⁰ 16]	- 20130208_Gel3	20170208_Gel4]	20130908_Gel1	[²⁰ ¥ ⁷ …]
		\sim			0.019			0.000	0.00	0.000	0.001							
		_	_				0.118		0	0 00			0.005	27824				1389.916
	Ц		\leq	\leq	0.120	0.075	0.049	0.626	0.40	0.627	0.003	0.002	0.001	28185				2250.424
	Н	\sim			0.089	0.029	0.157	0.322	1./6	1.580	0.002	0.001	0.002	2/818				985.450
			\sim			0.000	0.002	_ w				0.000	0.000					
H	Н			Ľ		0.037	0.002					0.002	0.000					
						0.013		0				0.001		28311				2729.689
				\checkmark			0.017		. 0	,			0.001					
				\checkmark			0.008		0	,			0.000					
		\checkmark			0.009			0.000	0.00	0.000	0.001							
		\checkmark			0.009		0.011	0.000	1,13	0.708	0.001		0.000	27687				843.773
	Ц		_	\leq			0.005	_	0)			0.000					
			\checkmark			0.088	0.097	00	0	· _		0.003	0.003					
	Ц						0.024		0	• • • •			0.001	2/925				595.560
	H				0.042	0.010	0.010	0 447	1.63	2.063	0.001	0.001	0.000	27756				065 401
					0.042	0.015	0.009	0.000	0.86		0.001	0.001	0.001	2//30				505.451
H	Н			v.	0.005		0.011	0.000	0.00	0.000	0.000		0.001					
	П					0.014		00				0.001						
						0.003		0 0		— 00		0.000		27351				1707.772
				\checkmark			0.007		0)			0.000					
										a				28322				483.776
			\checkmark			0.006		00		α α		0.000		28288				577.858
		_	\checkmark	\checkmark		0.549	0.643	0	•			0.012	0.008					
	Ц				0.015			0.000	0.000	0.858	0.001			27529				1663.788
	Ц	\sim		\sim	0.021		0.025	0.000	1.18	0.741	0.001		0.001	28109				2523.395
	H				0.088		0.088	0.000	. 0.000	0.847	0.003		0.003	27353				1283 002
		~			0.000		0.003	0.000	0.33	0.047	0.005		0.000	2///2				1203,302
H	Н						0.007						0.001	27316				405,301
	П				0.015			0.000	0.00	0.000	0.000							
			\checkmark	\checkmark	0.147	0.133	0.051	0.906	0.34	0.000	0.004	0.006	0.002					
					0.003		0.011	0.000	4.32	6 🚺 0.000	0.000		0.000					
				\checkmark			0.004		0				0.000					
										00				28337				181.914
				\checkmark			0.003		0				0.000					
		_		\checkmark			0.012		0				0.000					
	Ц	\leq			0.040			0.000	0.00	0.000	0.003			20145				564.040
										α				28146				1297 201
					0.124	0.010	0.020	0 1/2	0.214	0 795	0.002	0.001	0.001	2//59				1301.597
	H	\sim		\bowtie	0.134	0.019	0.029	0.145	0.21		0.003	0.001	0.001	20210				786 267
														27289				2034.414
H	Н				0.037	0.023	0.024	0.615	0.65	0.735	0.001	0.001	0.001	28112				1546.851
		<u> </u>	<u> </u>	<u> </u>														

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 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'.

20170908_Gel2_B7_ecoli_525 Cy5 ~									
to									
Group	Image	Transfer	Warp	Spots					
\checkmark	20170908_Gel1_Bx_grid_550 Cy3	\checkmark	٢						
	20170908_Gel1_Bx_ecoli_525 Cy5	\checkmark	Θ	1020 detected spots					
\checkmark	20170908_Gel2_B7_grid_550 Cy3_1	\checkmark	Θ						
	20170908_Gel2_B7_ecoli_525 Cy5			1075 detected spots					
	20170908_Gel2_B7_ecoli_525 Cy5 war	\checkmark	٢						
\checkmark	20170908_Gel3_Bm_grid_550 Cy3	\checkmark	Θ						
	20170908_Gel3_Bm_ecoli_525 Cy5	\checkmark	٢	883 detected spots					
\checkmark	20170908_Gel4_BK_grid_550 Cy3	\checkmark	Θ						
	20170908_Gel4_BK_ecoli_525 Cy5	\checkmark	٢	830 detected spots					
\checkmark	Fused Image using Union at 13_08_201	\checkmark	٢						

Figure 70:

'Transfer Spots from' before modification.

	20170908_Gel2_B7_ecoli_525 Cy5 ~									
to										
Group	Image	Transfer	Warp	Spots						
	20170908_Gel1_Bx_grid_550 Cy3		٢							
	20170908_Gel1_Bx_ecoli_525 Cy5		٢	1020 detected spots						
\checkmark	20170908_Gel2_B7_grid_550 Cy3_1		0							
	20170908_Gel2_B7_ecoli_525 Cy5			1075 detected spots						
	20170908_Gel2_B7_ecoli_525 Cy5 war	\checkmark	٢							
	20170908_Gel3_Bm_grid_550 Cy3		٢							
	20170908_Gel3_Bm_ecoli_525 Cy5		٢	883 detected spots						
	20170908_Gel4_BK_grid_550 Cy3		٢							
	20170908_Gel4_BK_ecoli_525 Cy5		٢	830 detected spots						
	Fused Image using Union at 13_08_201		٢							

Figure 71: 'Transfer Spots from' after modification.



O 2599 / 2343 (32%) Image Bounds: 2800 x 2400 Match Vectors: 0 Detected Spots: 0 / 1075

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.

	Y .			1 D122					_
E Light T	able 🗙	💶 Warpir	ng Setup	🗙 👩 Dual	View - 20	01709	08_Ge	1_Bx_	gri
Export Ed	dit Mark	Hide C	ancel N	ormalization	n Filter	Colu	mns	Labels	s
Filter	Filter						Mar	k	
²⁰ %V·]	- ² ¶ ¹⁷ ⊺	²⁹ 17]	²⁰¹⁷	²⁰¹⁷			Lid		
0.067	8.354	28260	835.029	1976.085			Thu	-	
0.003	0.379	28266	2720.182	1970.404			Nor	m 📗	
0.006	0.804	27433	1093.029	807.036			-		
0.002	0.263	27274	230.721	44.054			Can	cel	
0.672	83.838	28320	443.651	2154.124		\checkmark	%V		
0.084	10.526	27520	1212.037	911.769					
0.179	22.330	27954	1695.956	1538.213		\sim	V		
0.019	2.354	27865	2176.259	1383.048					
0.002	0.237	27282	1294.899	52.355			A		
0.024	2.984	28277	794.975	1997.033			Bgd		
0.048	5.938	27323	2147.297	257.921					
0.059	7.312	27970	1154.062	1536.839			Avg		
0.007	0.907	27340	494.192	410.746		1	ID		
0.048	6.043	28145	1187.338	1822.575		•	10		
0.023	2.857	27542	1609.546	948.886			Lab	el	
0.034	4.184	27997	2122.737	1572.227					
0.012	1.447	28113	2178.095	1770.685		~	Х		
0.003	0.315	28344	917.418	2277.309		\checkmark	Y		
0.004	0.543	28332	2160.807	2164.931			<u> </u>		
0.015	1.926	28109	2523.395	1762.239			Q		
0.086	10.779	27770	2051.896	1250.300			1.55	ala	
0.004	0.538	28015	2716.834	1595.094			Lab	eis	
0.013	1.589	27529	1663.788	916.052					
0.631	78 743	27704	1570 078	1284 008					

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 ycoordinate, 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	У	Mean y	Molecular weight [Da]		
1	976		56000		
3B	872	870	69293		
4B	869	070			
3C	1086	1001	42881		
4C	1077	1001			

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