

# ANALYZING 2D-ELECTROPHORESIS GELS

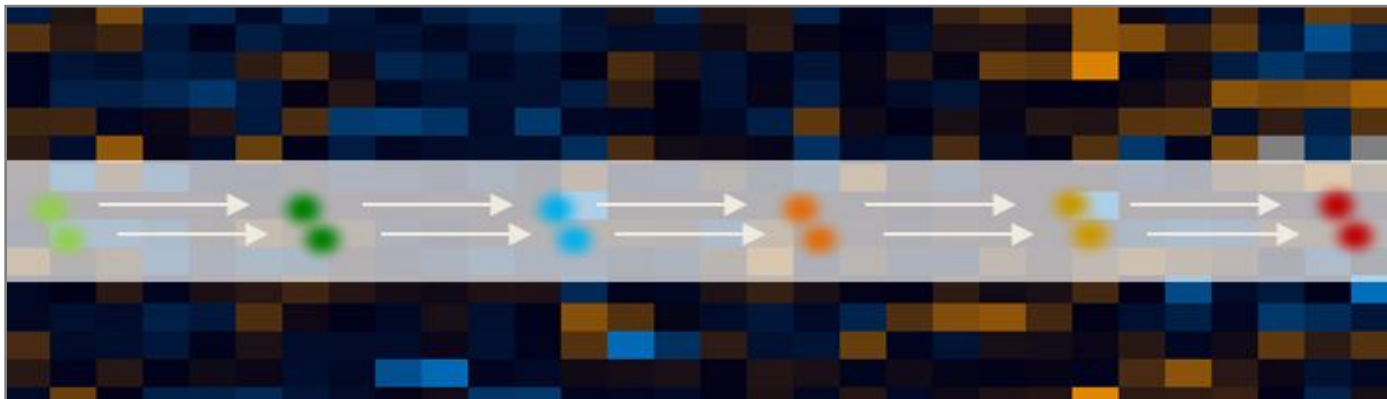


how to find interesting proteins in 5 steps

# Introduction



- 2DE Gel Analysis – what are we looking for?
  - interesting protein spots
    - different abundance caused by variation of experimental factor
  - reliable findings
    - statistical analysis
    - based on replicates
- Perfect basis for quantitative analysis: complete expression profiles



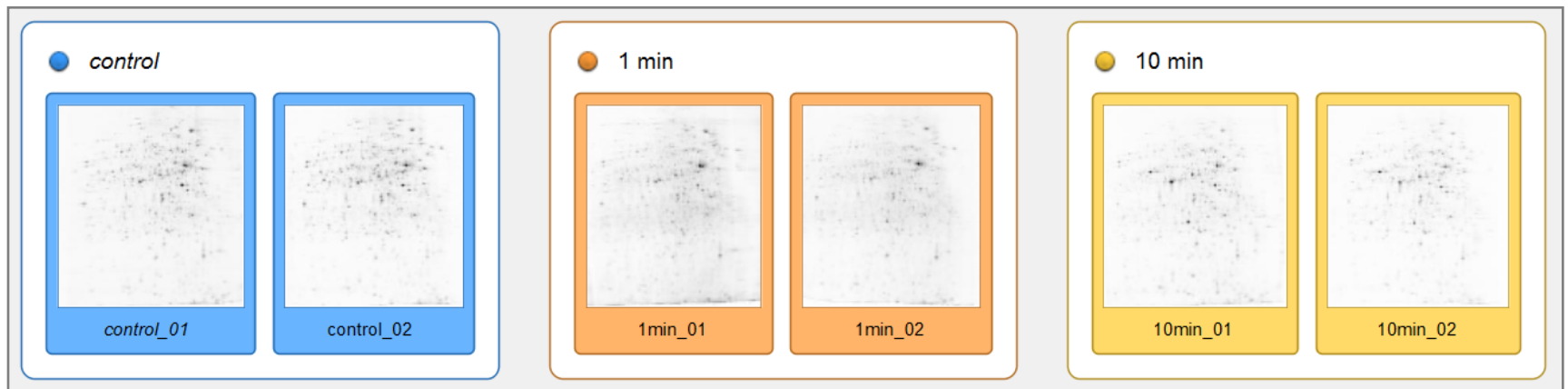
# Introduction



- 2DE Gel Analysis – why a challenge?
  - image analysis aspects
    - speckle artefacts
    - varying signal intensity
    - background signal
  - matching of spots across gels
    - differences in spot positions
    - differences in spot patterns
    - conflicting pairwise spot matching
  - missing spots
    - unique spot matching
    - statistical significance

# Step 1: Setup Project

- Create groups for replicates and import images (supported image file formats: tif, gel, jpg, png, inf, ...)



# Step 1: Setup Project

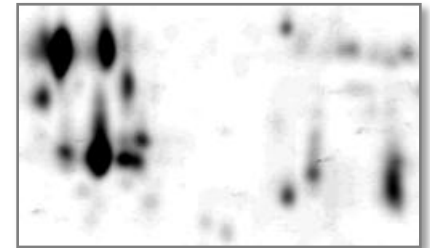


- Automatic image preparation during import

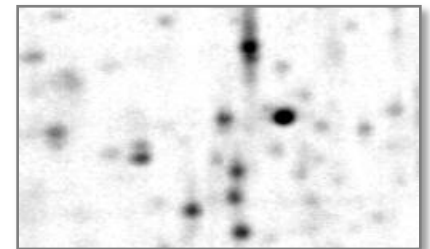
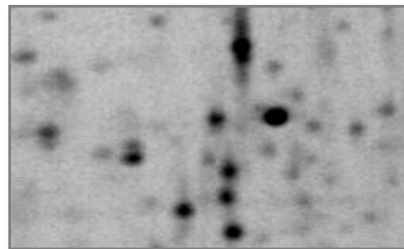
- filter for de-speckling



- contrast settings

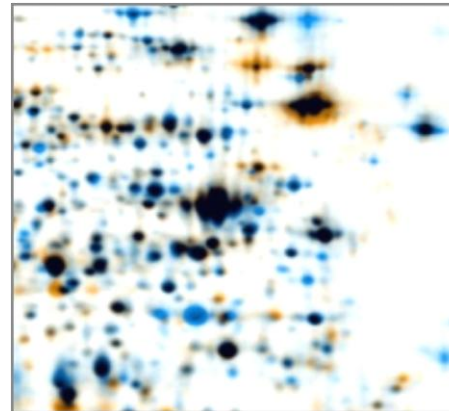
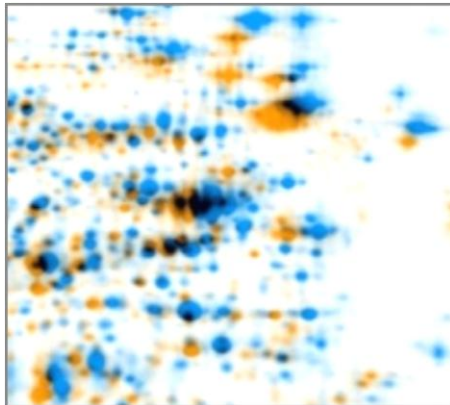


- background subtraction



# Step 2: Warp Images

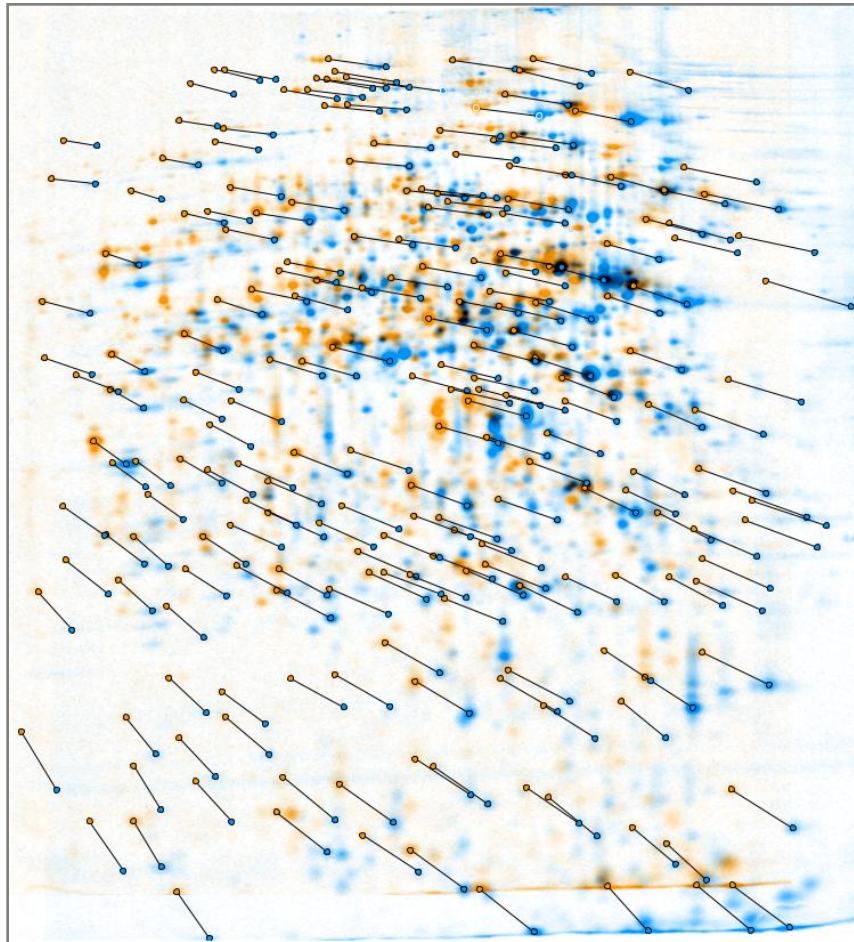
- Why image warping?  
Eliminate differences in spot positions!  
or, in other words:  
Compensate running differences between gels!



# Step 2: Warp Images

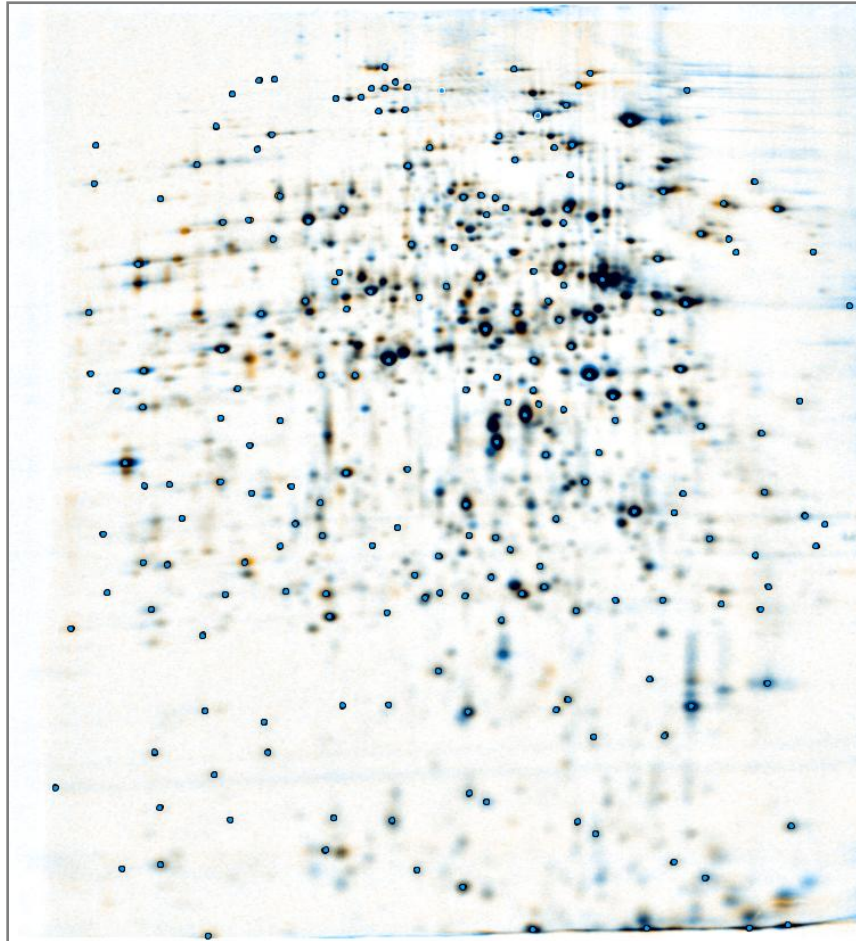


- Add match (warp) vectors automatically or manually:



# Step 2: Warp Images

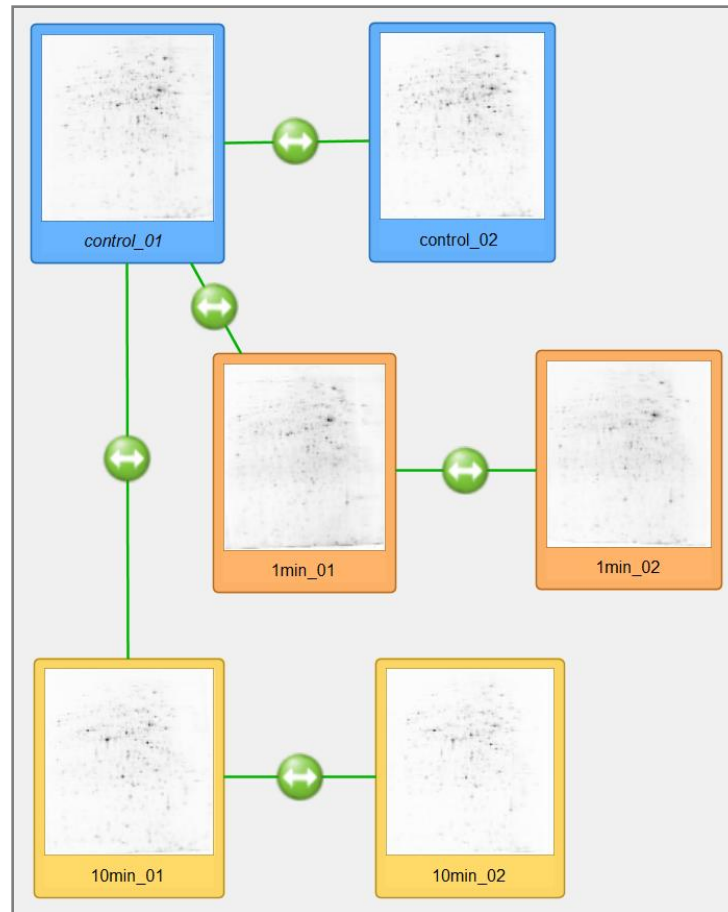
- Review warping result (and iteratively improve if necessary):





# Step 2: Warp Images

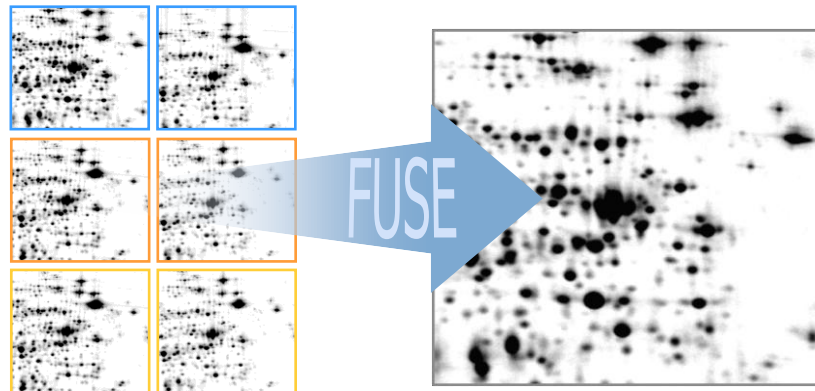
- Create complete set of warp relations for project



# Step 3: Detect and Quantify Spots

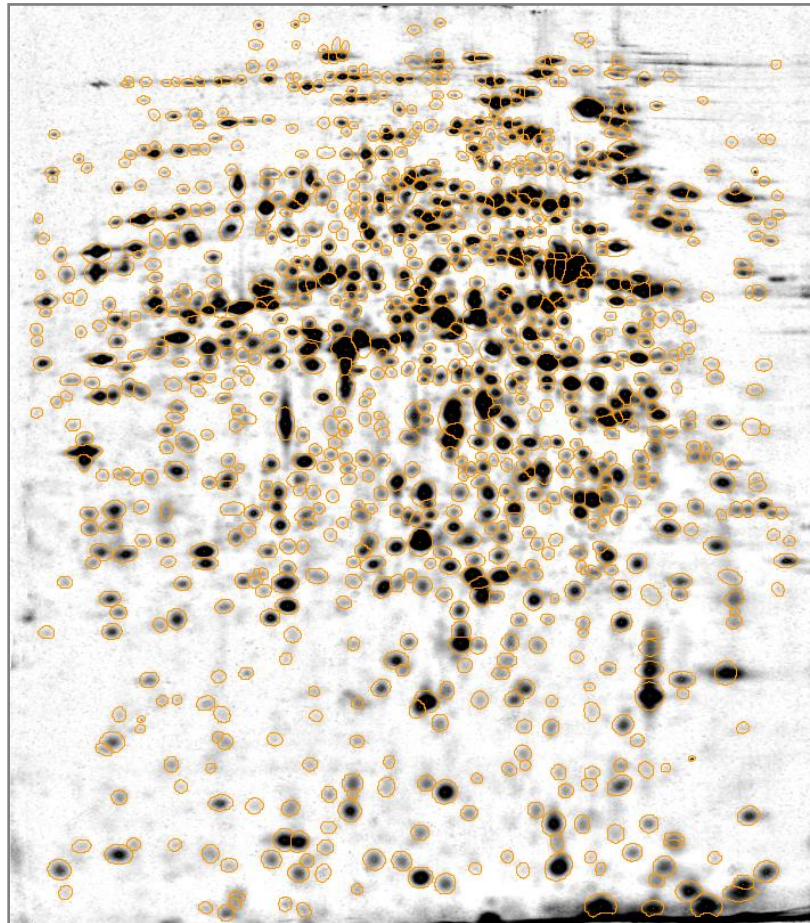


- Create a fused image containing all spots of the experiment:



# Step 3: Detect and Quantify Spots

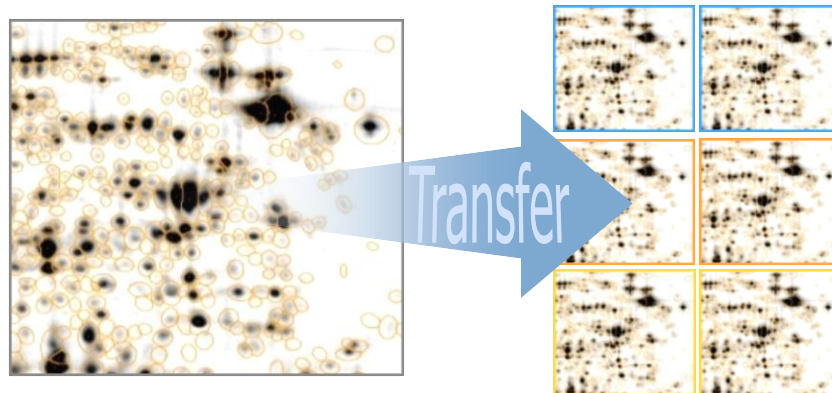
- Detect spots on the fused image:



# Step 3: Detect and Quantify Spots


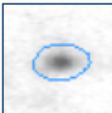
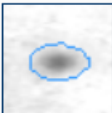
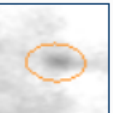
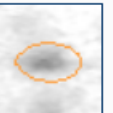
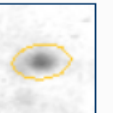


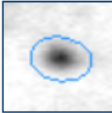
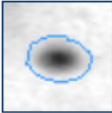
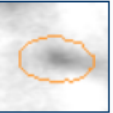
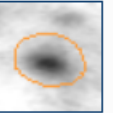
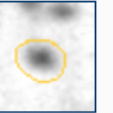
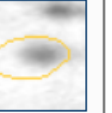
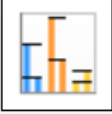
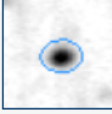
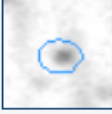
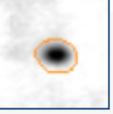
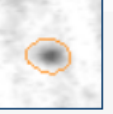
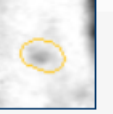
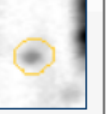


- Apply same spot pattern to whole experiment:



# Step 3: Detect and Quantify Spots

- Result: complete and unique spot matching!

Profile	Label(s) ↑	Spot ↓	control hide others		1 min hide others		10 min hide others	
			control_01	control_02	1min_01	1min_02	10min_01	10min_02
	Spot_01	13254						
	Spot_02	13307						
	Spot_03	13769						

- Avoided: different spot patterns!
- Avoided: conflicting pairwise spot matching!

# Step 3: Detect and Quantify Spots



- Result: expression profiles automatically quantified!

Label(s) ▾	Profile ▾	control hide others				1 min hide others						10 min hide others					
		control_01 ▾	control_02 ▾	Mean ▾	RSD ▾	1min_01 ▾	1min_02 ▾	Mean ▾	RSD ▾	Ratio ▾	p-value ▾	10min_01 ▾	10min_02 ▾	Mean ▾	RSD ▾	Ratio ▾	p-value ▾
Spot_01	13254	0.079	0.069	0.074	6.815	0.074	0.099	0.086	14.486	1.173	0.444	0.068	0.043	0.056	23.244	0.756	0.324
Spot_02	13307	0.146	0.158	0.152	4.062	0.129	0.284	0.206	37.612	1.354	0.560	0.085	0.084	0.085	0.457	0.556	0.008
Spot_03	13769	0.120	0.038	0.079	52.015	0.185	0.078	0.131	40.680	1.659	0.520	0.024	0.051	0.037	35.945	0.471	0.436

# Step 4: Analyze Expression Profiles

- Apply statistical tests like
  - HCL and HCL ST, KMC and KMS, PTM, to explore structures
  - t-test (numerous variations), ANOVA, 2-way-ANOVA, to find differences
  - PCA for quality control



# Step 4: Analyze Expression Profiles

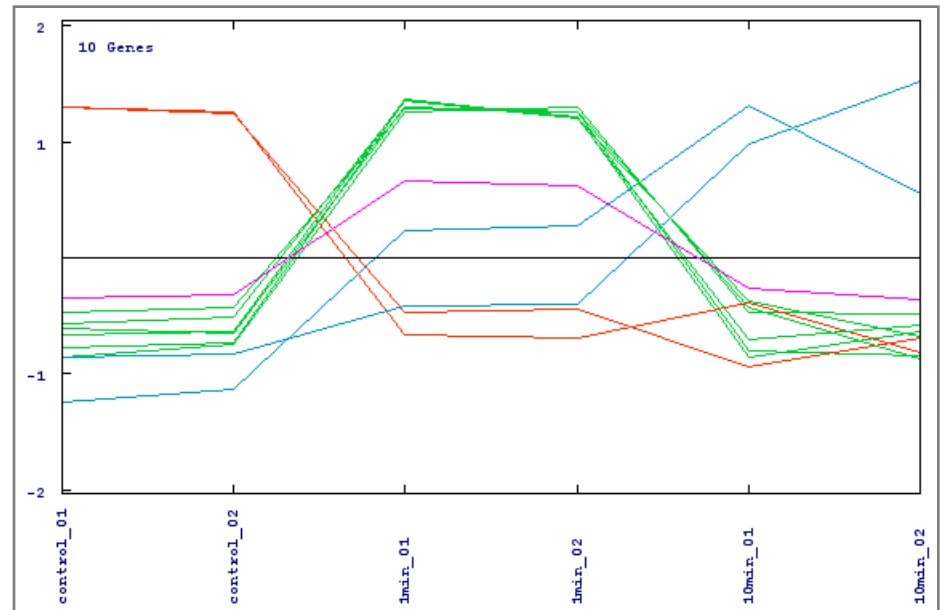
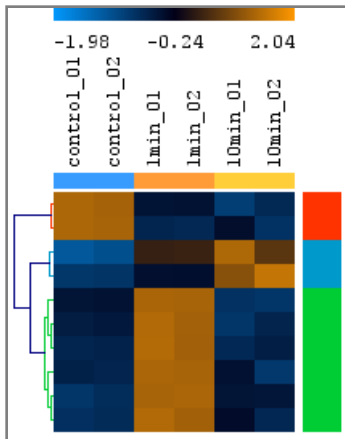


- Consider requirements like normal distribution of data and apply:
  - Nonparametric Tests like Wilcoxon / Mann-Whitney, Kruskal-Wallis, Mack-Skillings, und Fisher-Exact Test
- Be aware of multiple testing and avoid false positive findings by applying:
  - false discovery control (FDA)



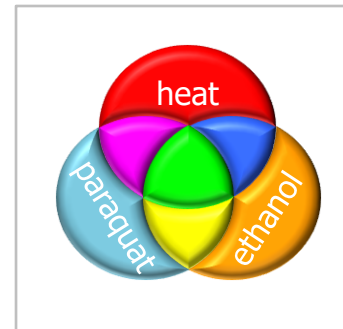
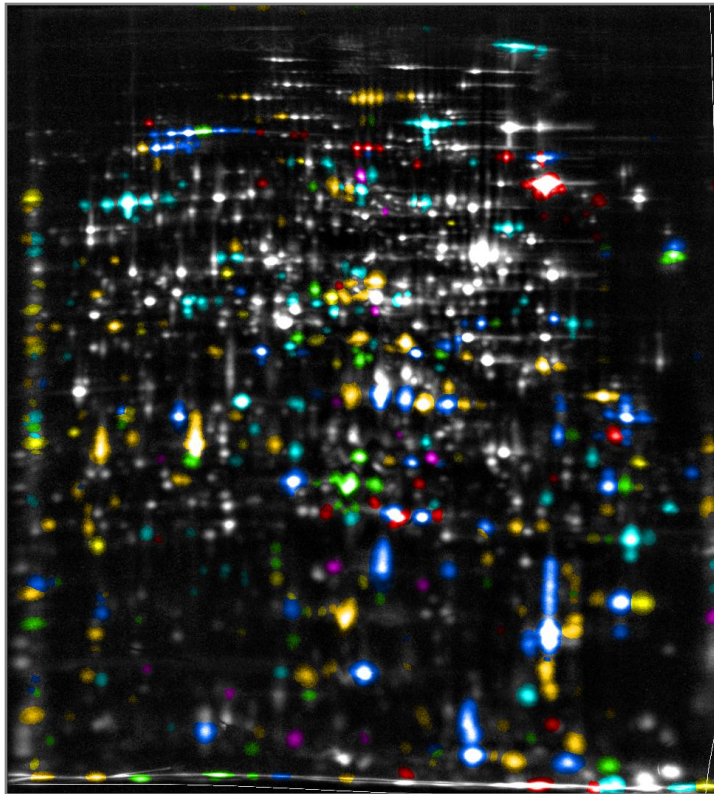
# Step 5: Present Results

- Prepare your findings for presentations or publications
- Prove that your findings are correct



# Step 5: Present Results

- Use Color Coding to create colorful presentations of your whole experiment



# Step 5: Present Results



- Export data as spreadsheets (to be read with commonly used software) as csv or xlsx
- Export images as presentation slides (to be read with commonly used software) as pptx or in standard image formats like png, jpg, tif
- Export reports in html (to be sent to advisors or to be published on websites)

**Delta2D Reports**  
for Project 'Demonstration'

Save all reports

▶ Project Properties

Report Index

Project Summary

Spot Album

Spot Quantities

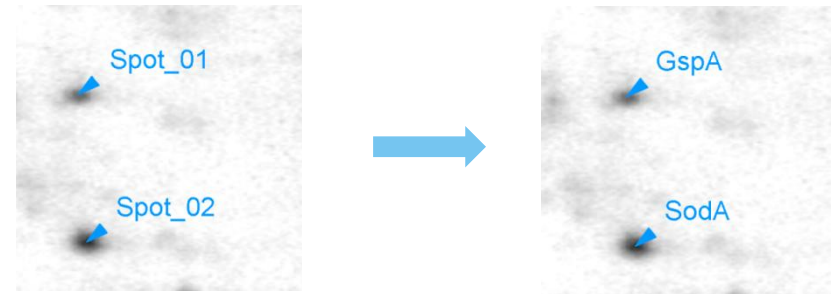
Labels

Image

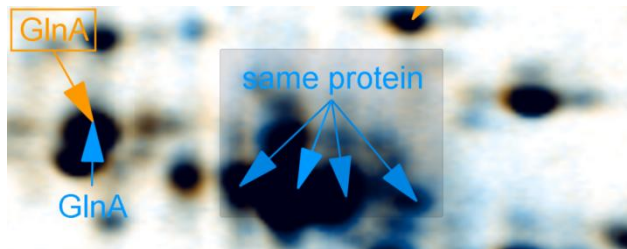
# Integrate MS and even more data



- Annotate spots
- Import MS identifications



- Manage, e.g. group annotations



- Add information from web databases like UniProt

Scout Data Editor

UniProt  
Label: GspA

AureoList Data Table GenBank GenoList Physicochemical properties UniProt

Input  
Query: GspA  
Organism: bacillus subtilis

Data

Organism: Bacillus subtilis (strain 168)  
NCBI Taxonomy: 224308  
Sequence: MRKDEIMHIVSCADDNYARHLGGMFVSLTNDQEREVKLYVIDGGIKPDKKRLLEETIKFGVPIEFLEVDTNMYEHAVESHITKAAYRISIPDLIKDESIRKMIYIDCDALVLEDISKLDLDAIAPYVAAVE DAGOHERLREMNVDTGKYFNSGIMIIDFESWRKQNIKTKVIFINEHPDEDFLVLDQDALNALYDQWYELHPRWNAQTYIMLKLKTPSTLLGRKOYNETIENFAIVHFCGGEKFPWNSNTKHPYRDEYFHYMSYTKWNTIGNFAINQ  
Isoelectric Point: 5.1072  
Molecular Weight: 33521.99  
Amino Acids: 286  
Function:

# References



- Software package:  
Delta2D
  - [www.delta2d.com](http://www.delta2d.com)
  
- Developing company and vendor:  
DECODON GmbH
  - [www.decodon.com](http://www.decodon.com)