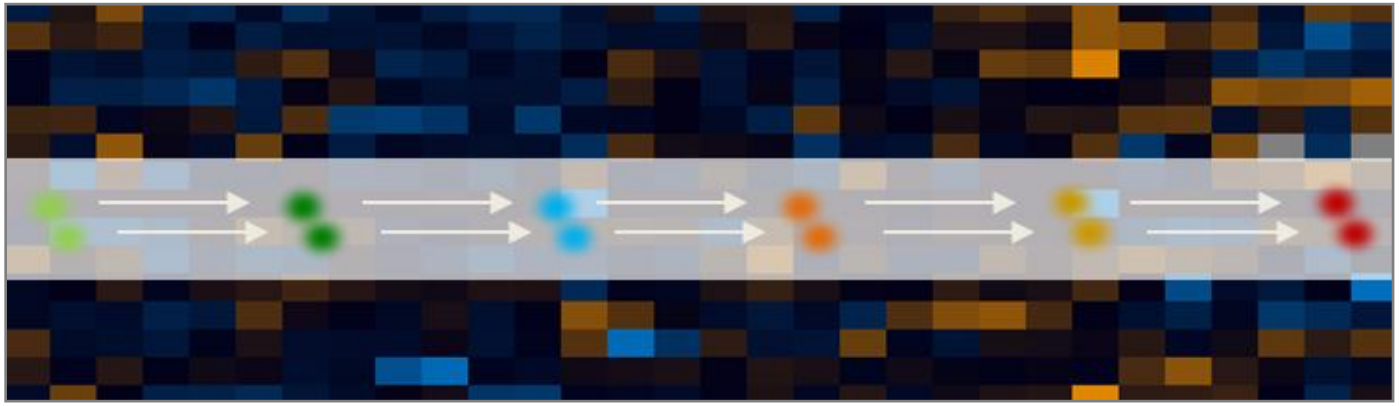
ANALYZING 2D-ELECTROPHORESIS GELS

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

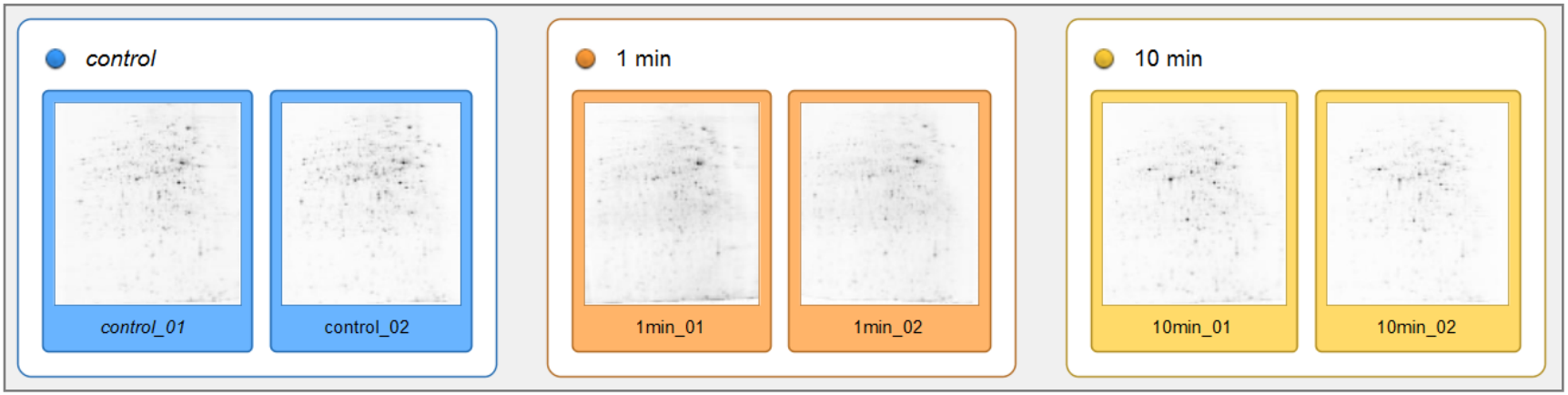


Introduction

- 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

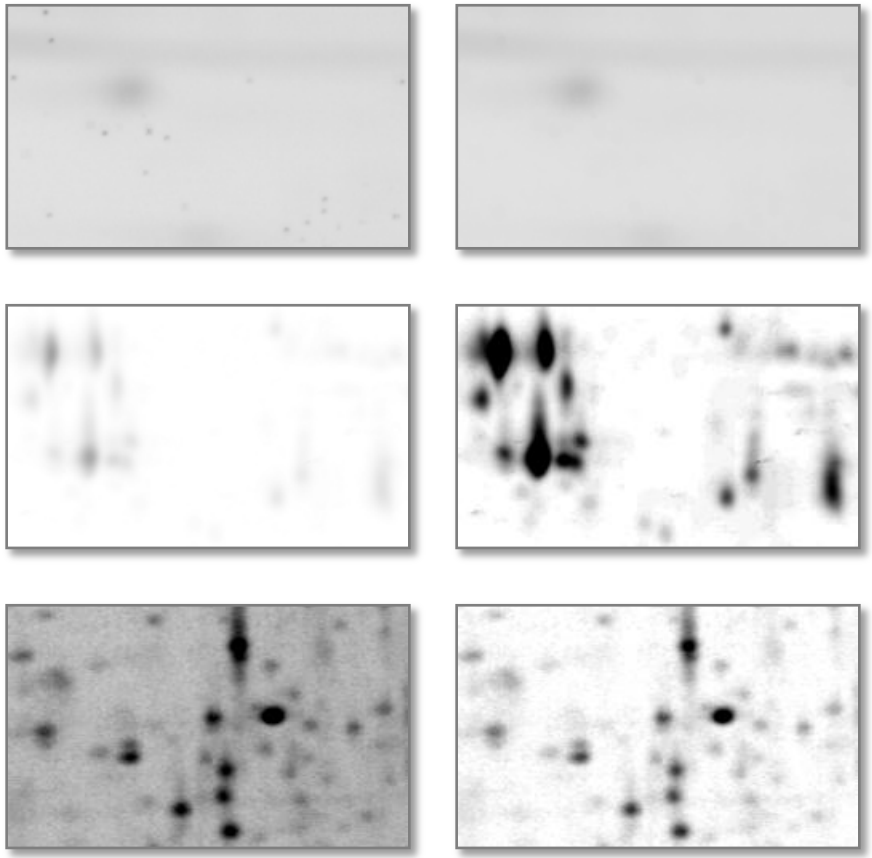
Step 1: Setup Project

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



Step 1: Setup Project

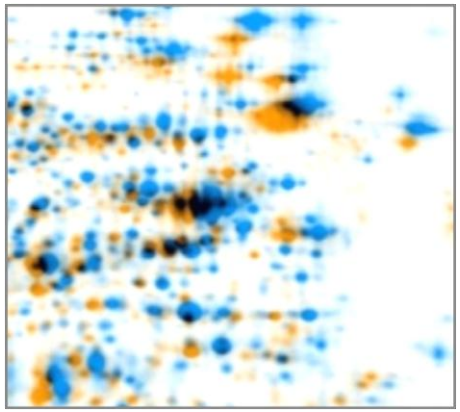
- Automatic image preparation during import
  - filter for de-speckling
  - contrast settings
  - background subtraction

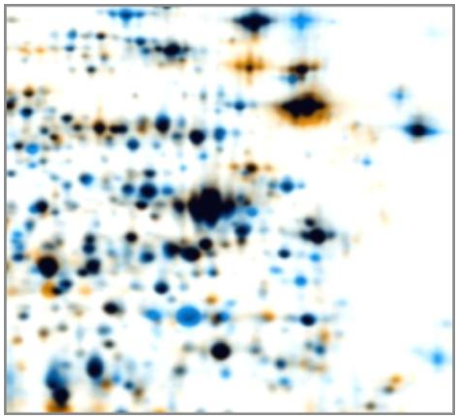


Step 2: Warp Images

- Why image warping?
  Eliminate differences in spot positions!

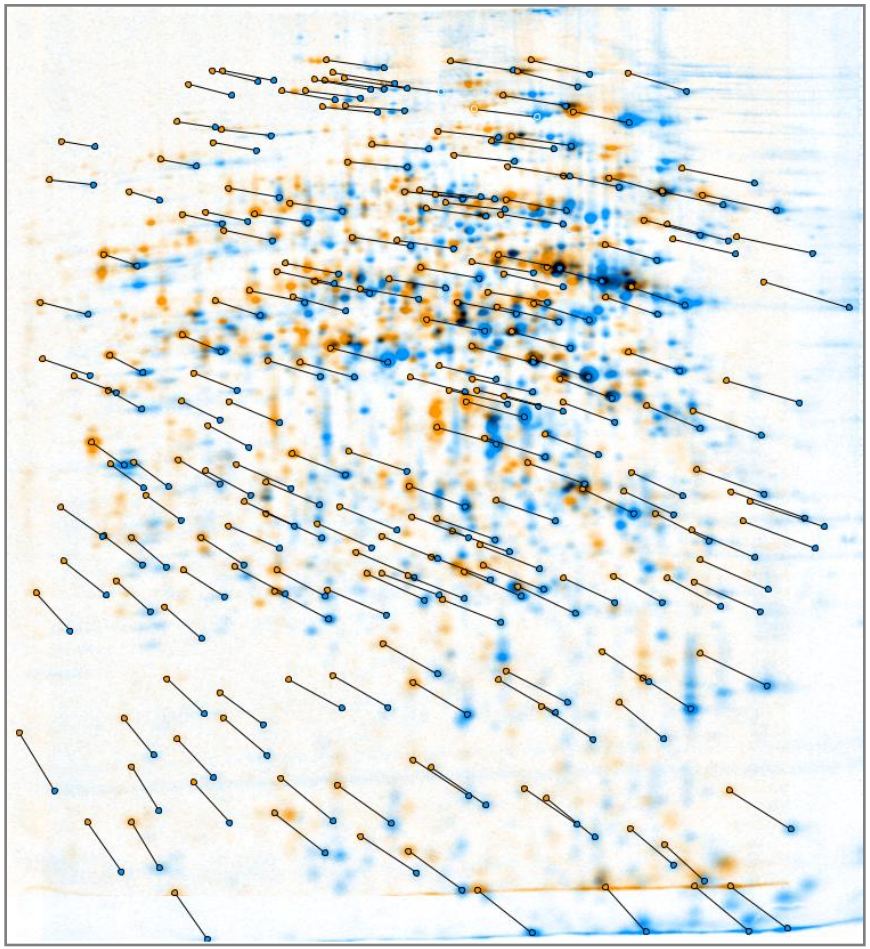
  or, in other words:
  Compensate running differences between gels!





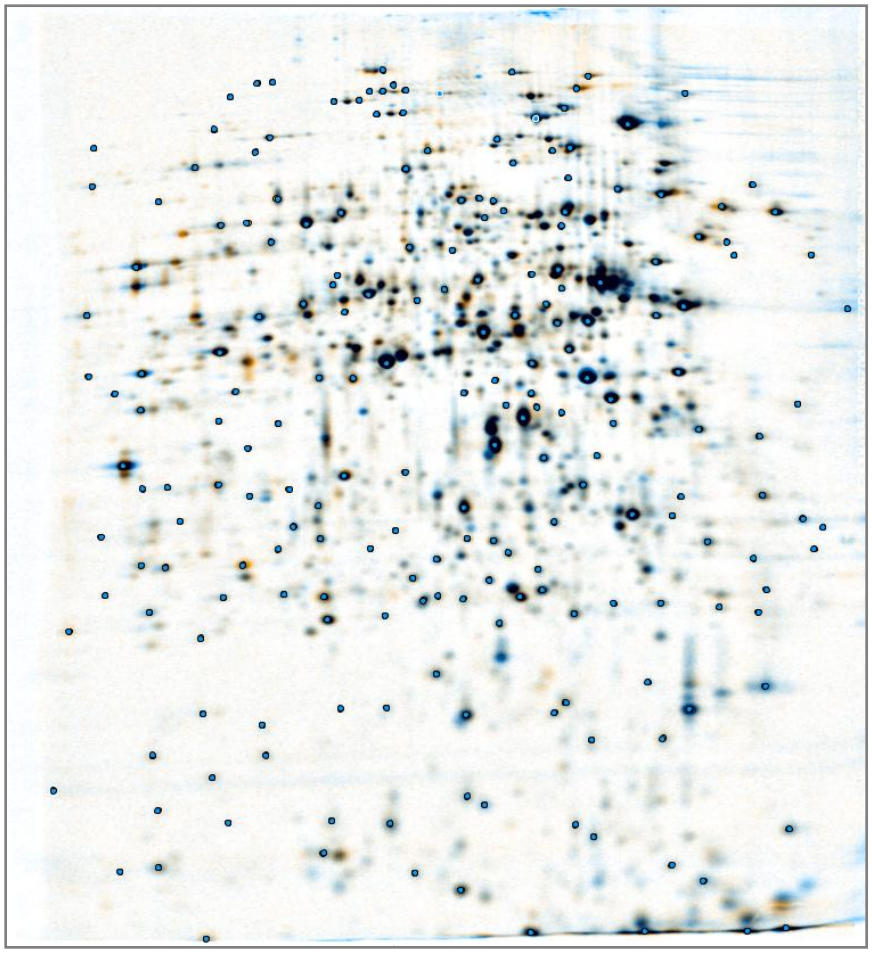
Step 2: Warp Images

- Add match (warp) vectors automatically or manually:



Step 2: Warp Images

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

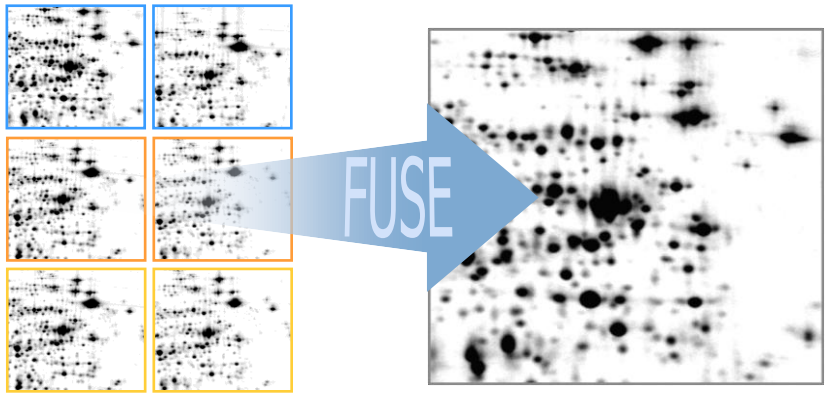


Step 2: Warp Images

- Create complete set of warp relations for project

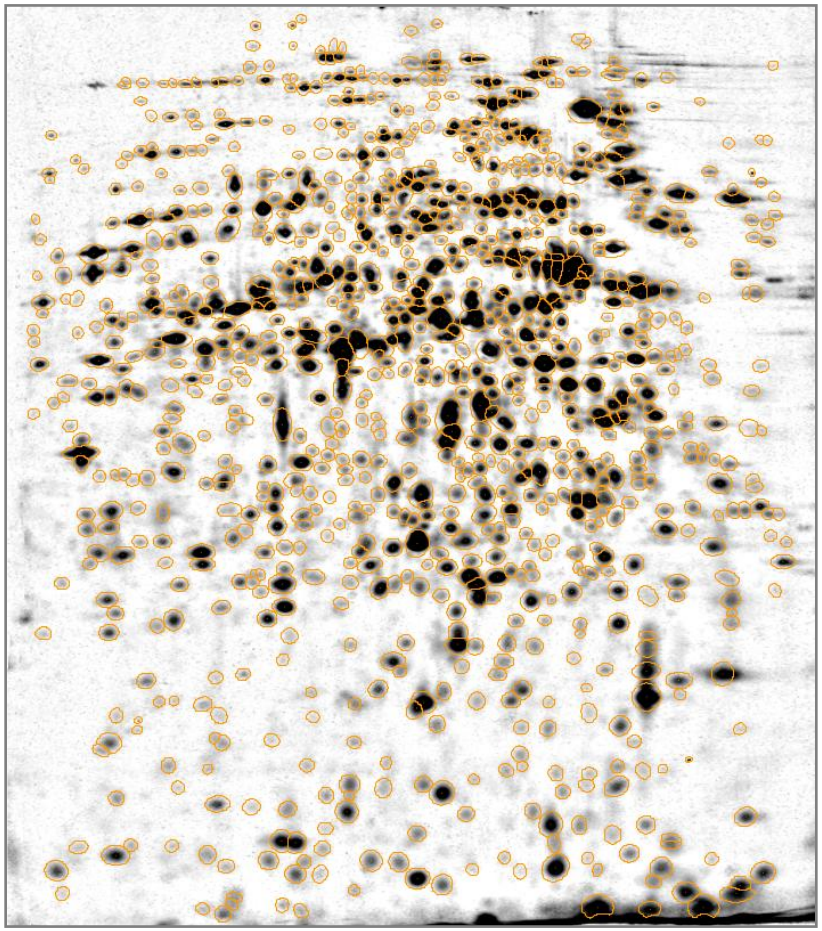
Step 3: Detect and Quantify Spots

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



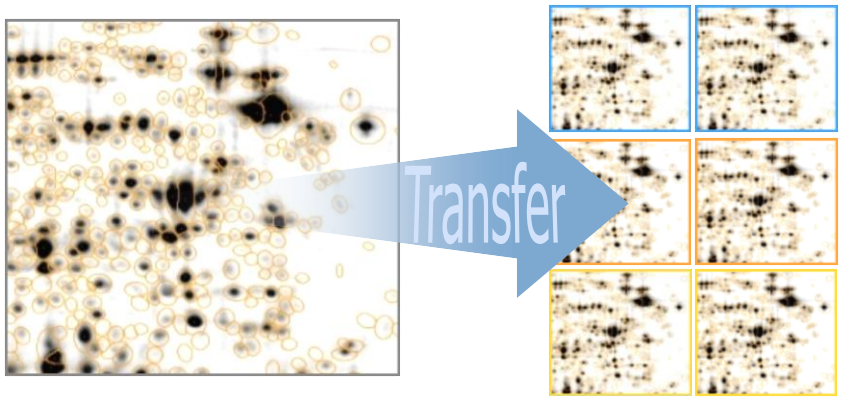
Step 3: Detect and Quantify Spots

- Detect spots on the fused image:



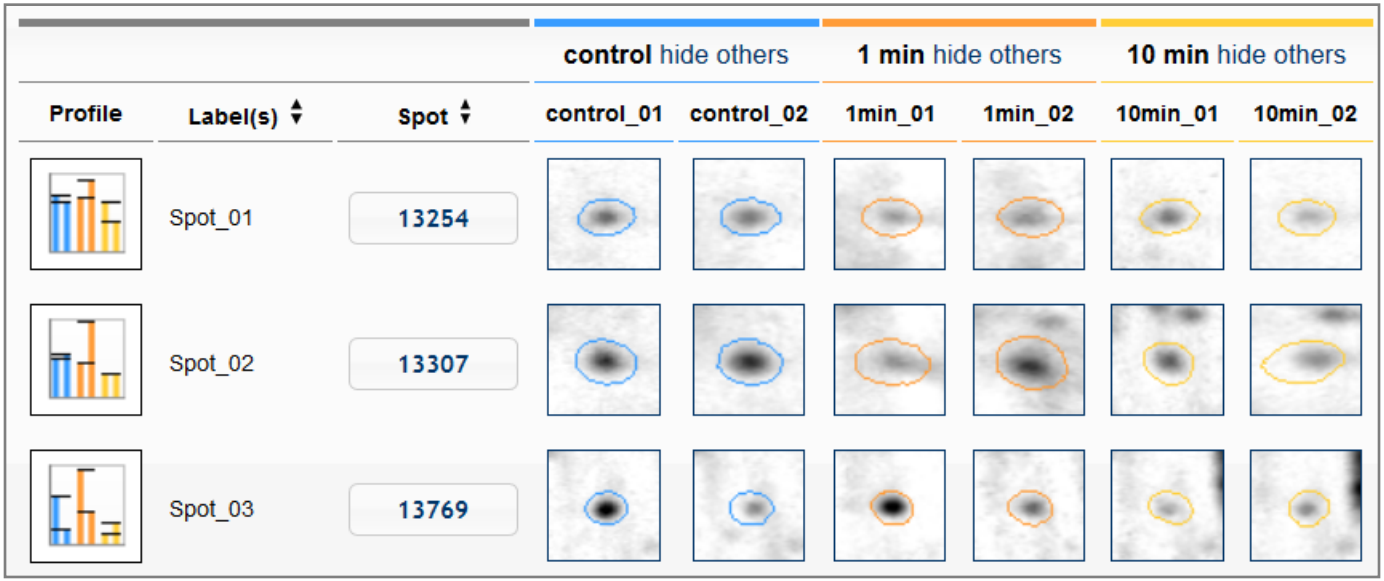
Step 3: Detect and Quantify Spots

- Apply same spot pattern to whole experiment:



Step 3: Detect and Quantify Spots

- Result: complete and unique spot matching!



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

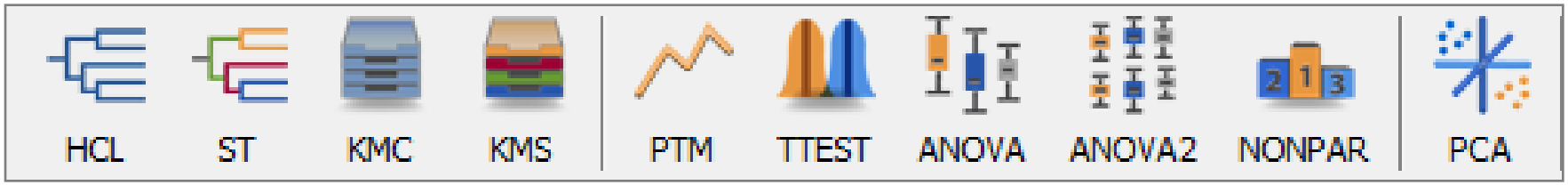
Step 3: Detect and Quantify Spots

- Result: expression profiles automatically quantified!

		control hide others				**1 min** hide others						**10 min** hide others					
Label(s)	**Profile**	**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

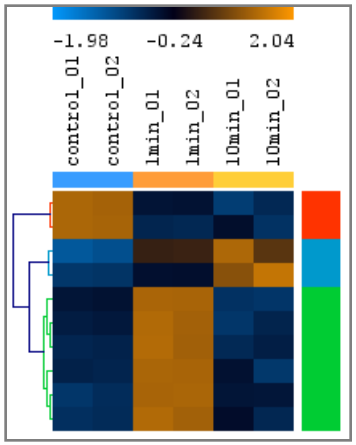


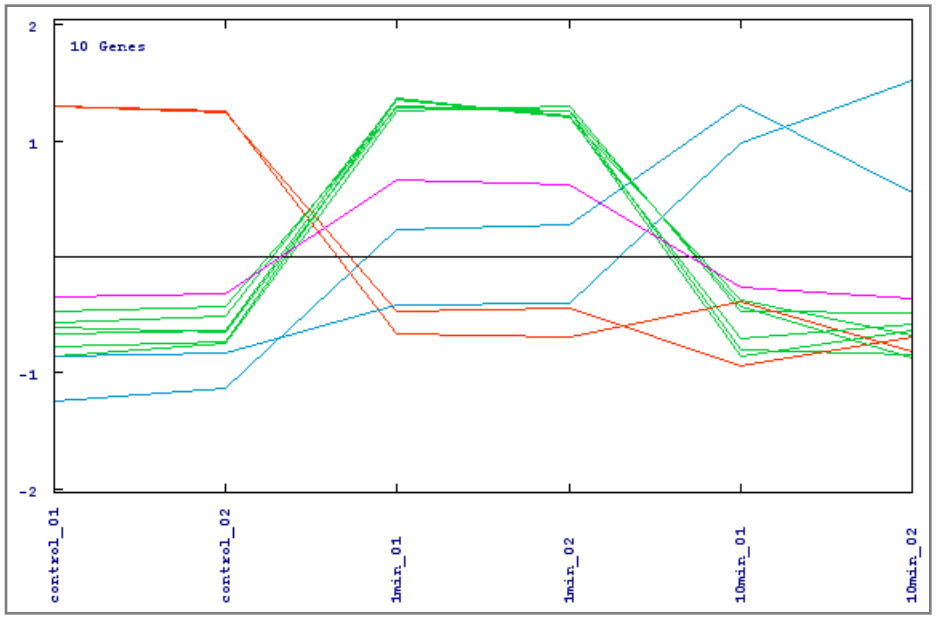
Step 4: Analyze Expression Profiles

- 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

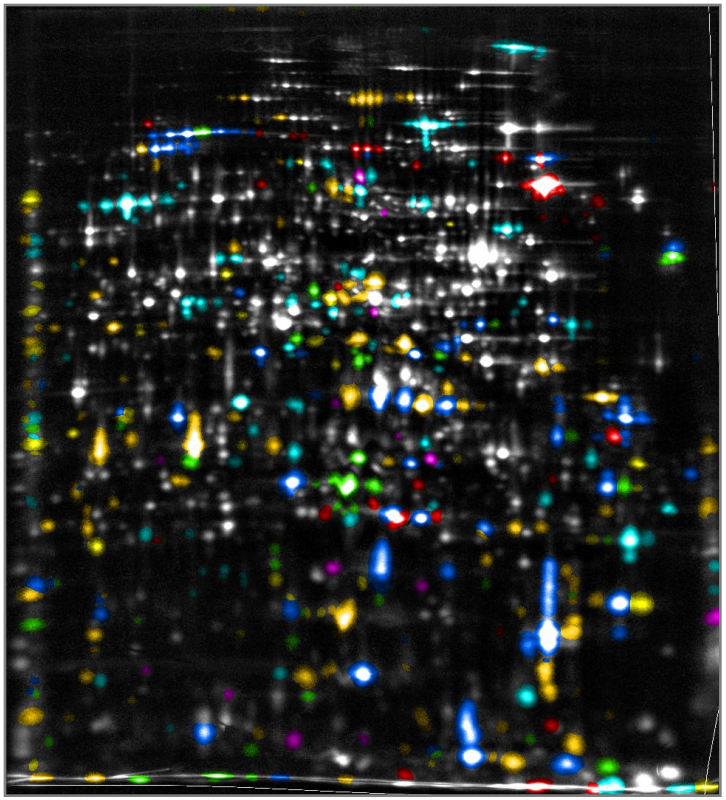
- Prepare your findings for presentations or publications
- Prove that your findings are correct

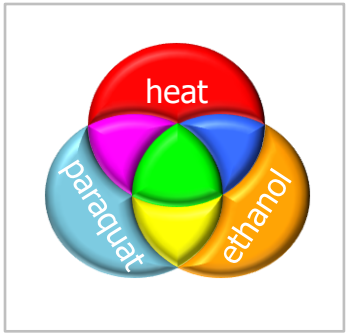




Step 5: Present Results

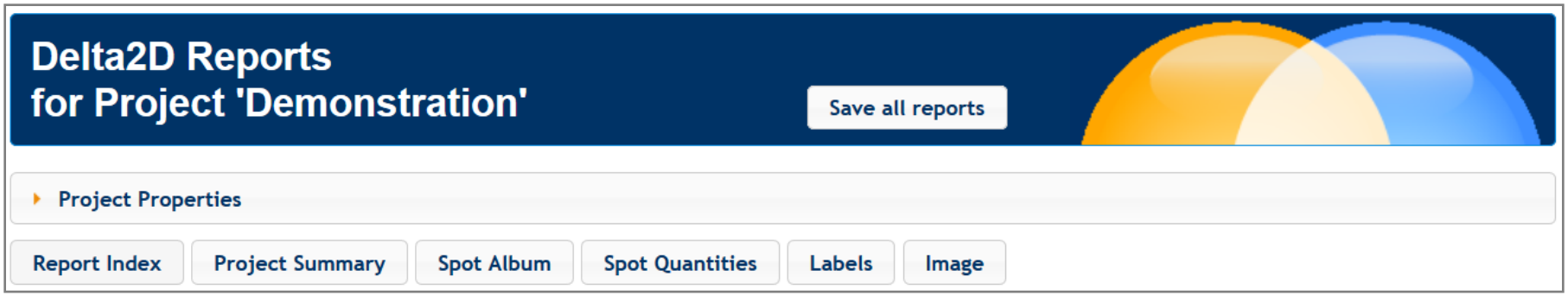
- Use Color Coding to create colorful presentations of your whole experiment





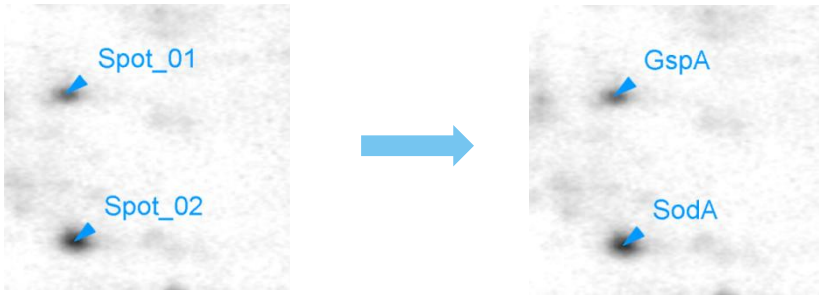
Step 5: Present Results

- 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)

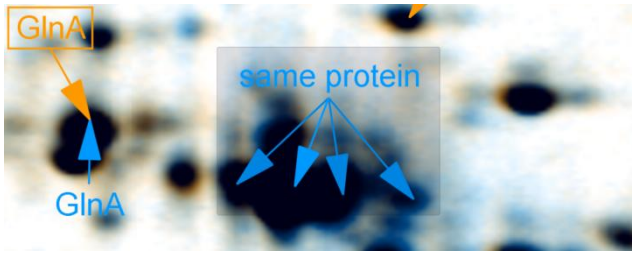


Integrate MS and even more data

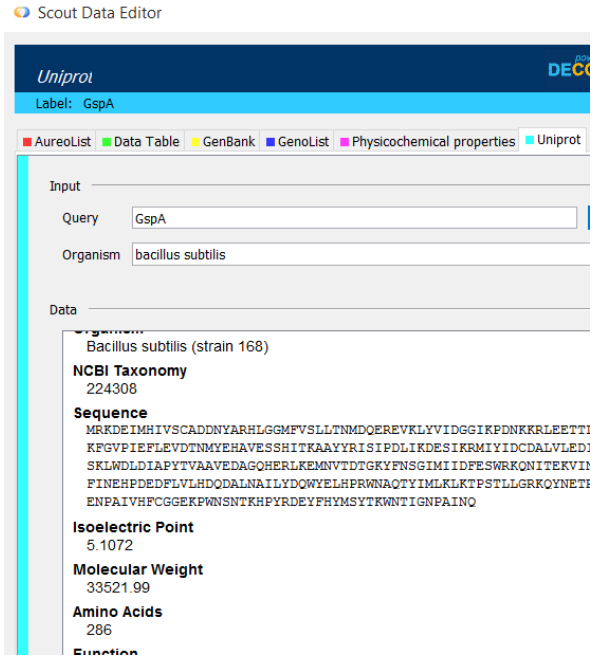
- Annotate spots



- Import MS identifications
- Manage, e.g. group annotations



- Add information from web databases like UniProt



References

- Software package:
  Delta2D
  - www.delta2d.com

- Developing company and vendor:
  DECODON GmbH
  - www.decodon.com