SameSpots 2D DIGE –
the limitations of current 2D image analysis

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A Nonlinear Dynamics Group presentation

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Major New Releases of 2008

- Progenesis SameSpots V3.0 (later V3.1)
- Prodigy SameSpots V1.0
- TotalLab TL100 and TL120 V2008
- TotalLab 21CFR part 11 module
- Coming soon! – Progenesis LC-MS

SameSpots Technology Vs. “Traditional” Approach

SameSpots Approach

- Simple, highly automated linear Workflow
- Near pixel-level alignment before spot detection.
- Common spot map applied to all gels simultaneously
- 100% matching of all spots across every gel in experiment
- No missing values
- Editing on one gel is applied to all others
- Common spot boundary minimises quantification variance.

Traditional Approach

- Complex, manual workflow
- Spot-level alignment by “warping” after spot detection
- Spots detected independently on each gel
- Typically many spot mis-matches even after editing.
- Many missing values typical
- Editing of spots on every gel necessary
- Different spot boundary on every gel – large quantification variance.

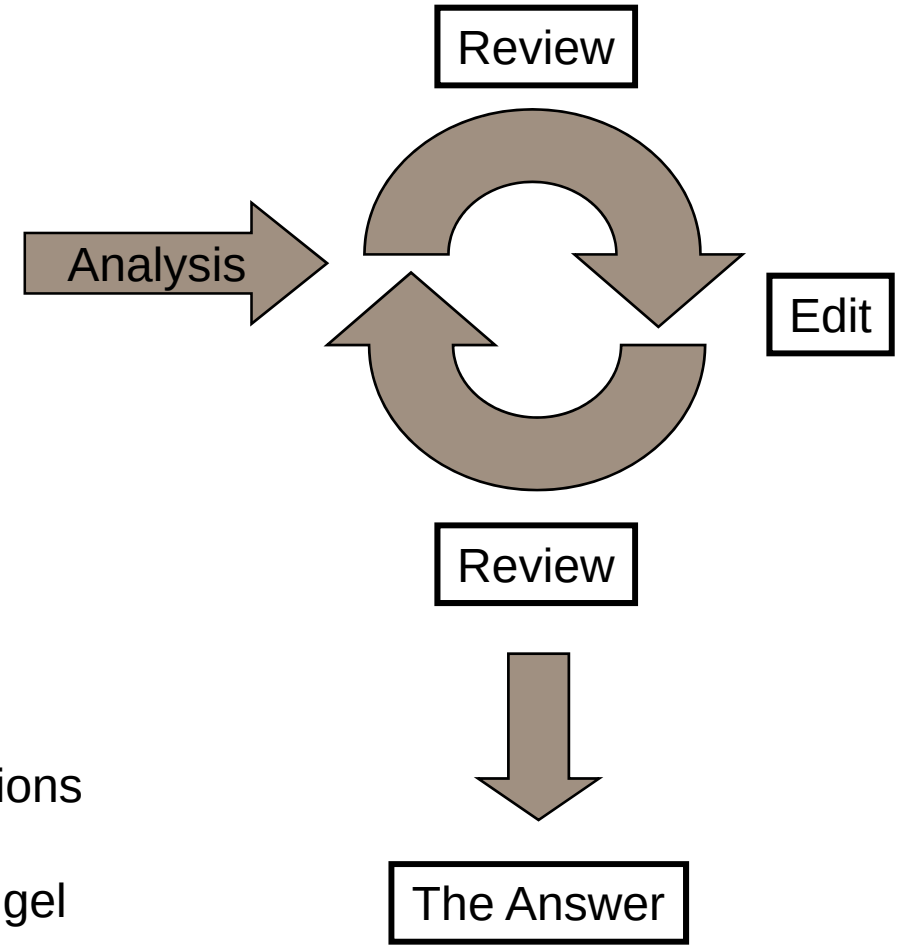
Traditional Workflow

Set your experimental design

Analyse the gels

Review the results
- ✓Check warping
- ✓Check spot detection
- ✓Check matching

The Pain
- ➢Redo warping
- ➢Editing on multiple gels
- ➢Redo matching
- ➢Redo background subtractions
- ➢Redo normalisation
- ➢Update average/reference gel



Nancy Kendrick, Ph.D., Kendrick Laboratories
The differences between SameSpots and traditional 2D analysis are profound. The old way required extensive spot editing and manually correcting the matching, which took a very long time. With SameSpots, the analysis is much less time-consuming.

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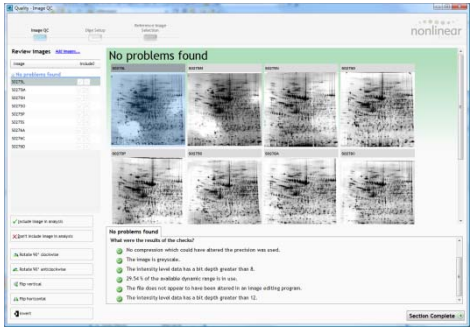
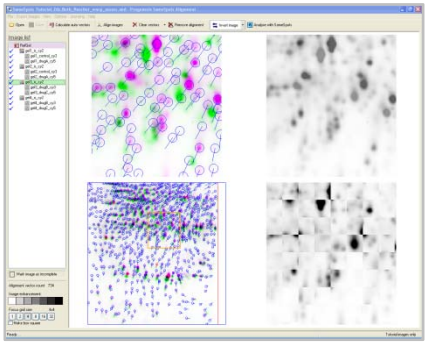
Progenesis SameSpots Workflow

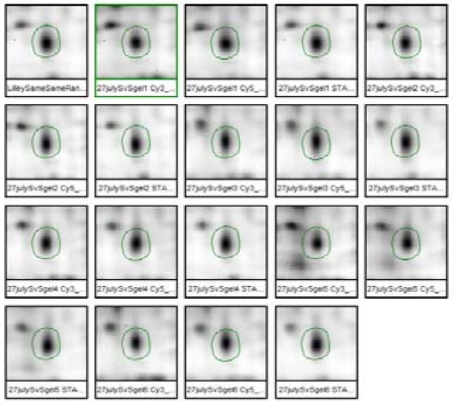
QC Images → **Align Images** → **Analyse Images and apply SameSpots** → **Validate Hits**



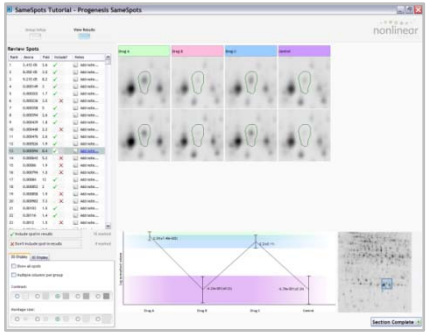
QC review of your images allows identification of problems before you start your analysis.



DIGE or single stained gels are aligned before detection to correct for positional variation.



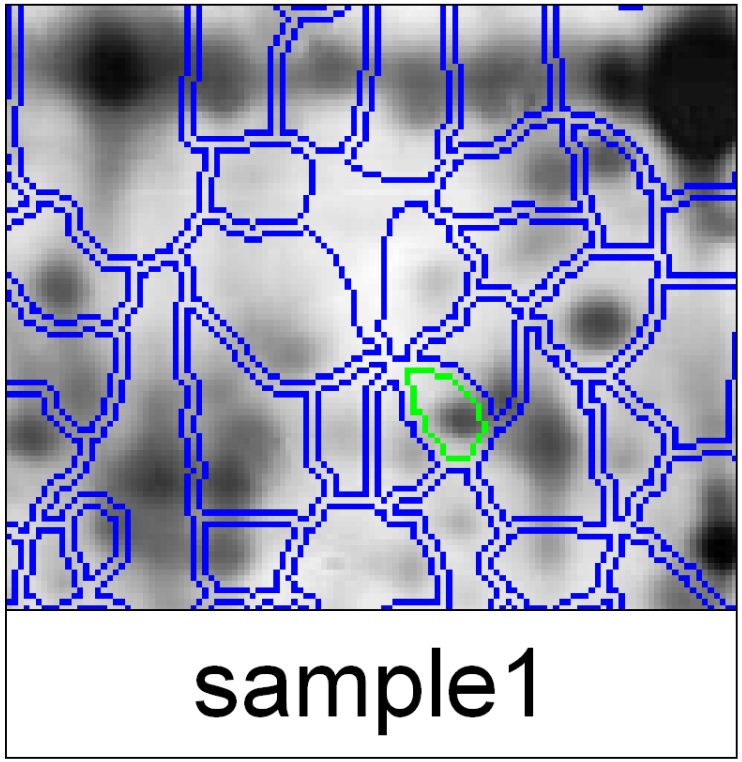
Images are analysed and identical spot outlines are transferred to all gels.

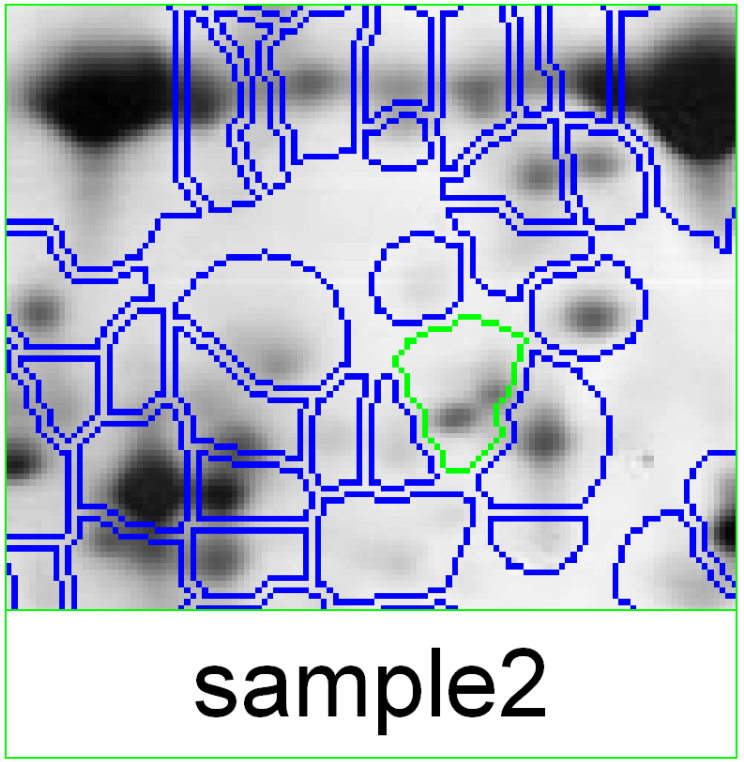


Statistics are performed automatically. Simply work down the list and validate hits.

Common Problems with 2D Image Analysis

- Detection and Editing

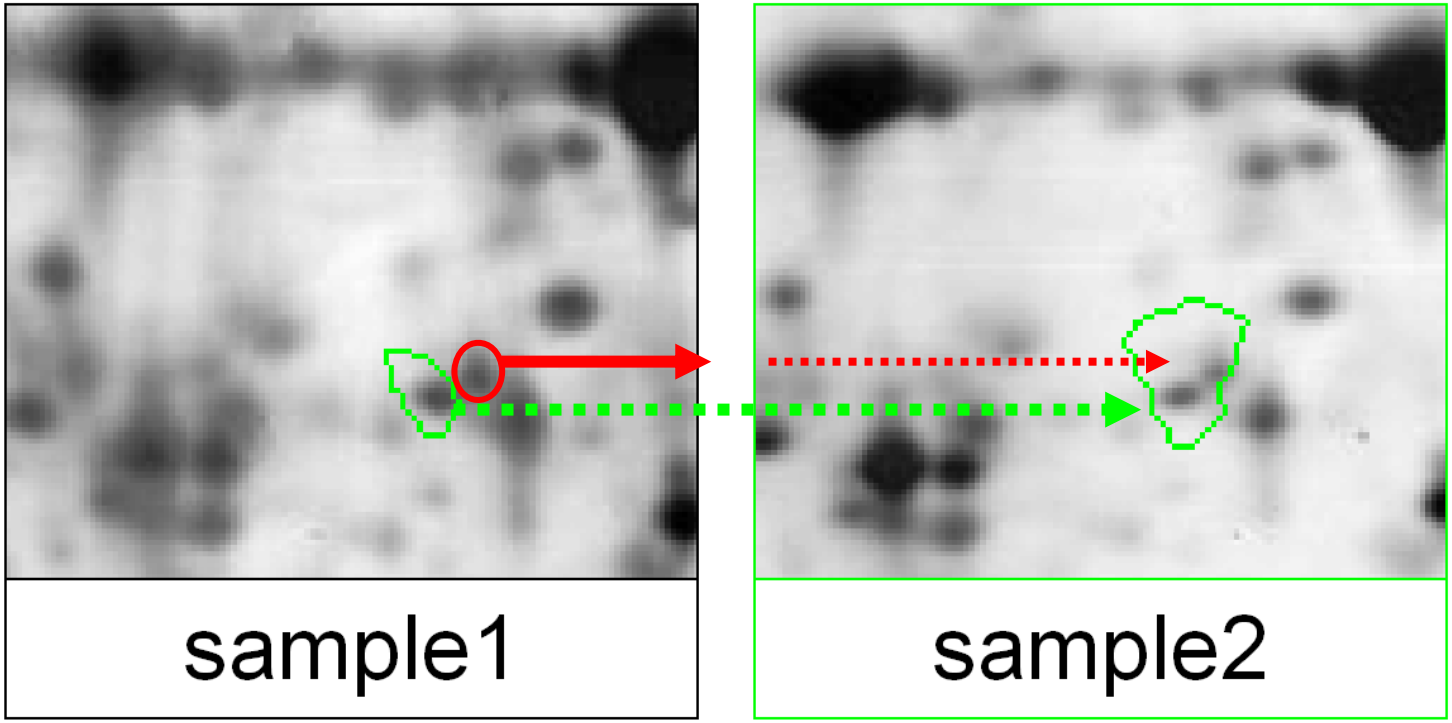




> **Dr. Jean Louis Dacheux, INRA Nouzilly, France**
> I am a fan of the new SameSpots approach which allows me to easily realign my gels prior to analysis. SameSpots also reduces my hands on time while increasing the quality and the level of confidence in the results produced. I am very excited to be pioneering this technology.

Common Problems with 2D Image Analysis

- Detection and Editing
- Matching inaccuracies



Dr. Peter Ashton, University of York, UK

The changes we have noted in our analysis have been very encouraging. The ability to perfectly align images dramatically improves matching performance. This enables highly consistent and reliable results to be obtained from all our 2D data sets, even those that previous image analysis packages were unable to accurately quantify.

Common Problems with 2D Image Analysis

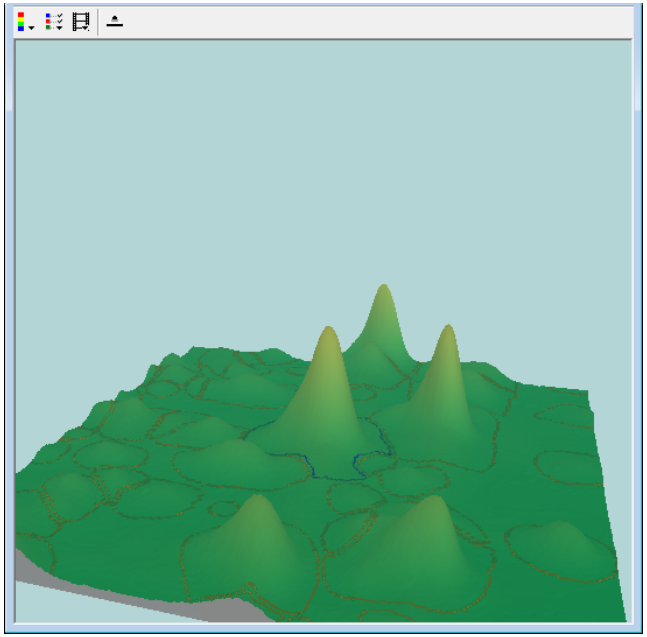
- Detection and Editing
- Matching inaccuracies
- Statistics – “Missing Values”

Control 2a	Control 2b	Control 2c	Treatment 2d	Treatment 2e	Treatment 2f	
Norm. Vol.	Norm. Vol.	Norm. Vol.	Norm. Vol.	Norm. Vol.	Norm. Vol.	t-test (p)
0.018	0.03	0.008	0.026	-	0.008	0.425
0.021	0.026	0.007	0.036	0.007	0.014	0.273
0.012	-	0.003	0.011	0.015	0.016	0.673
0.018	0.026	-	0.035	0.023	-	0.0627
0.049	0.037	0.032	0.009	0.006	0.014	0.0017
0.004	0.002	-	0.12	0.058	0.13	0.032

“**David S. Gibson, PhD, Arthritis Research Group, Queen’s University Belfast**
With SameSpots we are able to highlight significant changes between patient subgroups with a higher degree of confidence, as previously overlooked missing values are now retained within the data set.”

What are the goals of 2D expression studies?

- Find the biologically significant expression changes
  - Reliably
  - Objectively
  - Quickly
  - Statistically relevant



> **Professor Mark S. Baker, CEO, Australian Proteome Analysis Facility Ltd (APAF)**
> SameSpots delivers an alternative starting point for 2D image analysis - offering significant improvements in image matching. The benefit of reliably matching spots across all gel images from the start means you can quickly focus your research efforts on investigating the few spots showing statistically valid changes, which are indicative of potentially interesting expression profiling changes.

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Let’s look at…

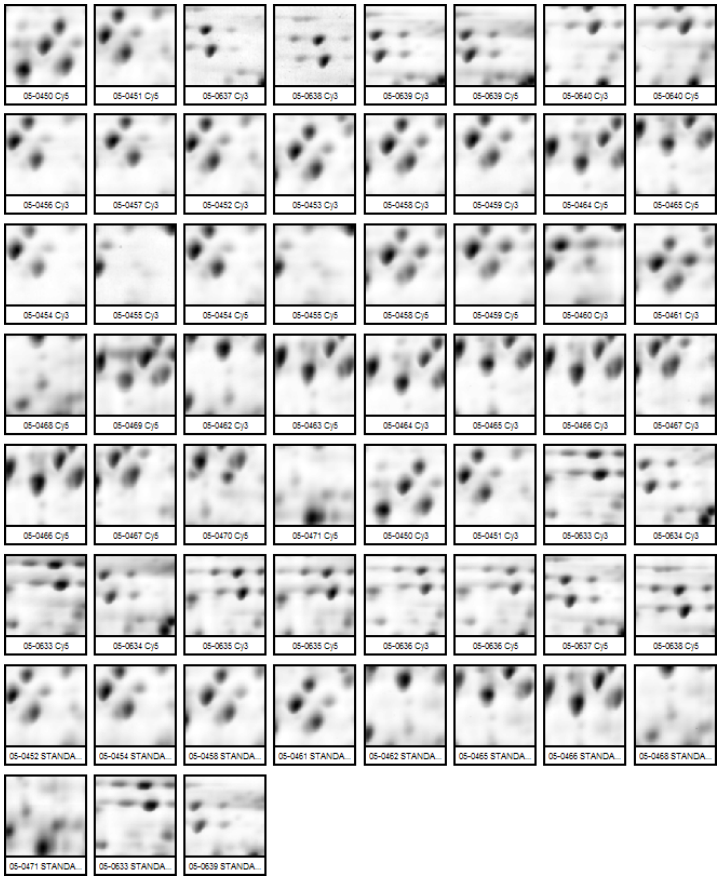
Progenesis SameSpots V3.0

2D Image software- tutorial experiment

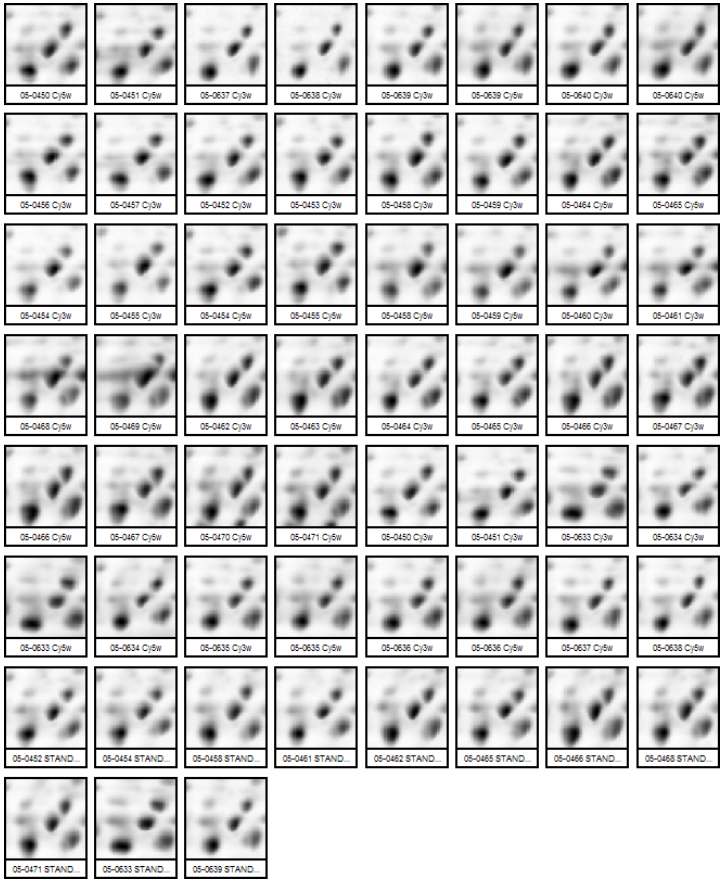
So how does SameSpots Technology Solve this Problem?

Perfect alignment is the KEY to SameSpots

From this…



..to this



> **Hans Voshol & Jan van Oostrum, Novartis Institutes for BioMedical Research**
> We thought this level of alignment was impossible!"

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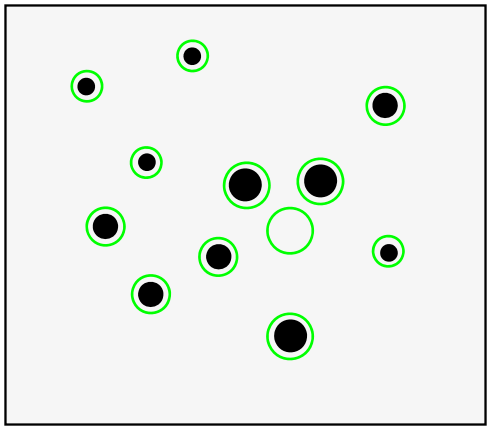
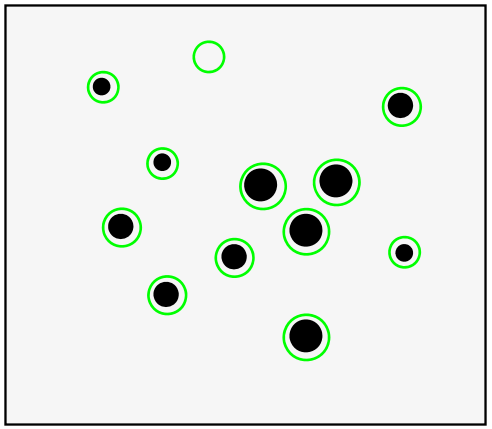
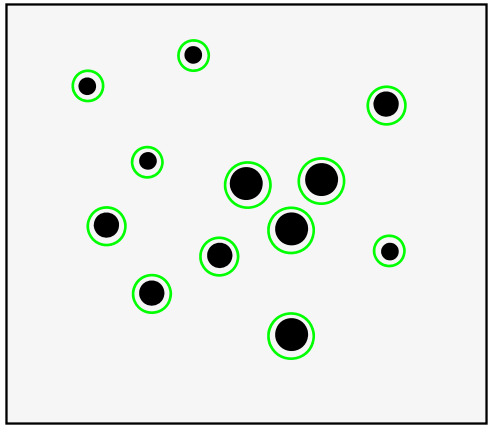
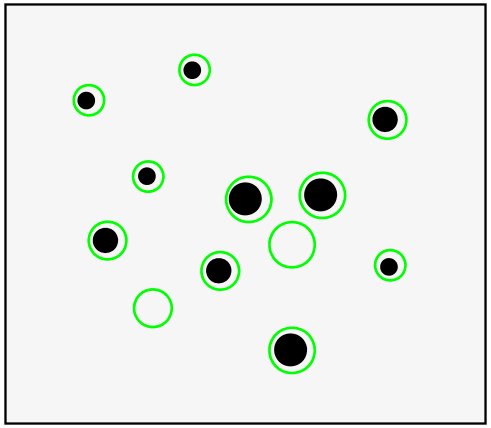
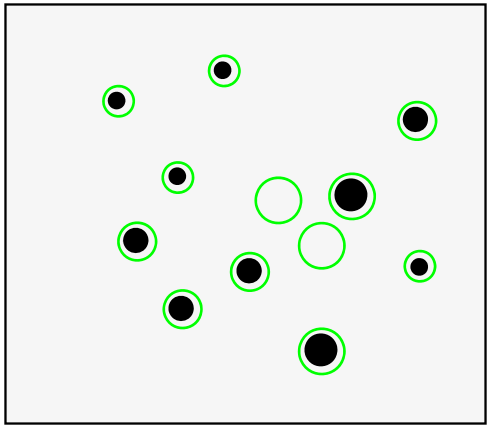
A simplified example of SameSpots detection









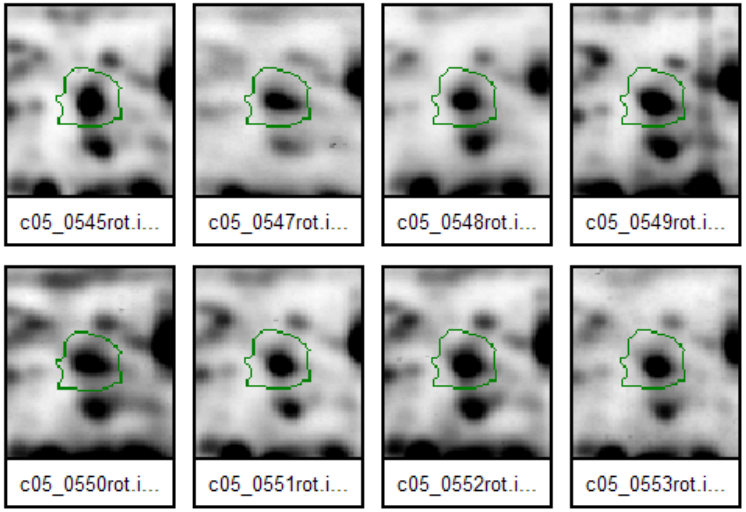


100% matching, no missing values and a complete dataset

Gel (replicate)

Spot

Ref. Spot	c05_0545...	c05_0547...	c05_0548...	c05_0549...	c05_0550...	c05_0551...	c05_0552...
	Spot	Spot	Spot	Spot	Spot	Spot	Spot
2201	2201	2201	2201	2201	2201	2201	2201
2202	2202	2202	2202	2202	2202	2202	2202
2208	2208	2208	2208	2208	2208	2208	2208
2222	2222	2222	2222	2222	2222	2222	2222
2230	2230	2230	2230	2230	2230	2230	2230
2233	2233	2233	2233	2233	2233	2233	2233
2235	2235	2235	2235	2235	2235	2235	2235
2236	2236	2236	2236	2236	2236	2236	2236
2240	2240	2240	2240	2240	2240	2240	2240
2247	2247	2247	2247	2247	2247	2247	2247
2253	2253	2253	2253	2253	2253	2253	2253
2256	2256	2256	2256	2256	2256	2256	2256
2258	2258	2258	2258	2258	2258	2258	2258
2265	2265	2265	2265	2265	2265	2265	2265
2266	2266	2266	2266	2266	2266	2266	2266
2267	2267	2267	2267	2267	2267	2267	2267
2269	2269	2269	2269	2269	2269	2269	2269



> **Doug Hinerfeld, Ph.D., Technical Director, BioMachines Applied Proteomics Group**
> A well recognized limitation of 2D image analysis is the ability to perform advanced statistical tests on analyzed data. By implementing SameSpots in our analysis workflow, we are achieving complete matching across an experiment, with no missing values, so we can have confidence in any statistical tests we choose to perform.

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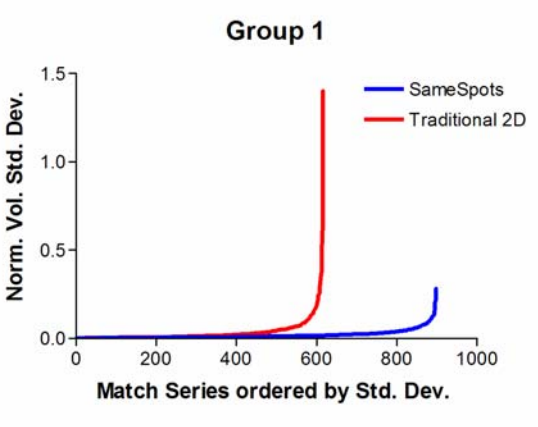
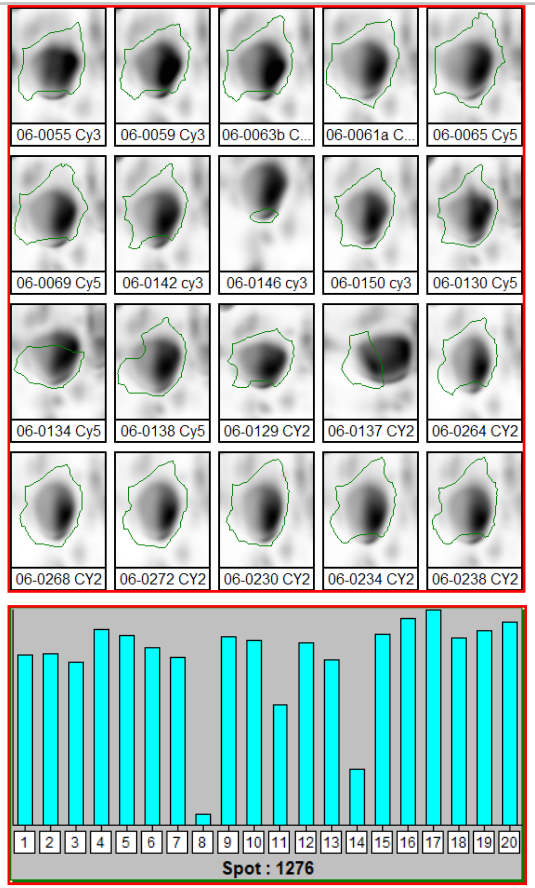
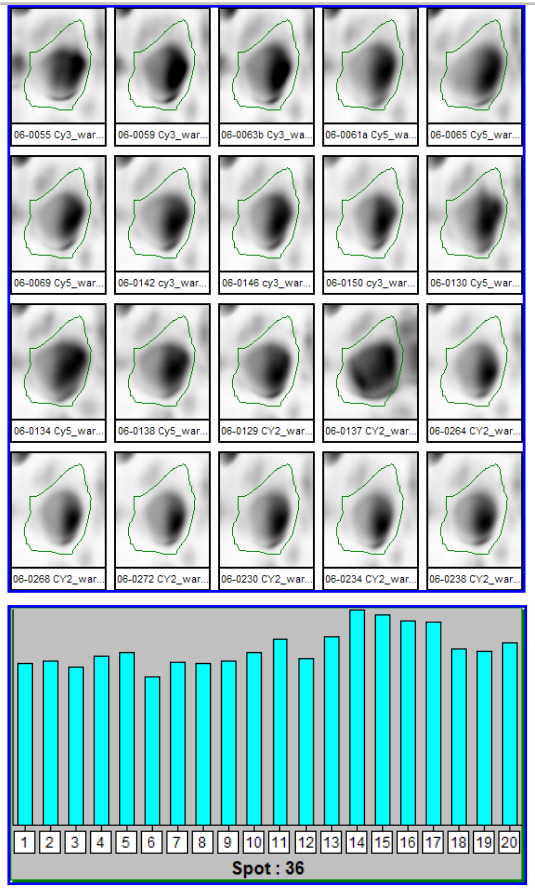
Reduced variability when using SameSpots





Traditional Analysis



SameSpots Analysis

Ashutosh Gupta, Rice University

SameSpots is much advanced and ahead of its competitors. It has reduced the analysis time to minutes from hours. Its statistical power is unmatched; with the ability to find same spot on all of the gels on exactly same location, problem of missing values from the gels is gone. SameSpots is definitely better than previous versions and is much advanced than its competitors.

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SameSpots Benefits

SameSpots Approach

- **Speed:** analysis of up to 20 gel set in 2 hours.
- **Objectivity:** more than 90% correlation of top 100 significant spots.
- **Statistical Power:** No missing values and low quantification variance = high stat. power
- **Simplicity:** Linear workflow and high level of automation = simple analysis!

Traditional Approach

- Analysis can take many hours, days, or even weeks!
- Much user dependence due to manual editing and matching stages.
- Missing values and high quantification variance = low statistical power.
- No linear workflow, cycle of reviewing, editing, re-warping, re-matching etc. = complex analysis!