

Progenesis SameSpots – Workflow Guide

Image QC

Add the images to analyse – either the tutorial or your own images. Several quality checks are performed and a report issued for each image. Images can be cropped, rotated and flipped prior to analysis if required.



DIGE Setup

If you are analysing a DIGE experiment, you need to group your images into the gels they originated from. The software should automatically identify the Internal Standard (Cy2), but you can change this if necessary before moving to the next stage.



Reference Image Selection

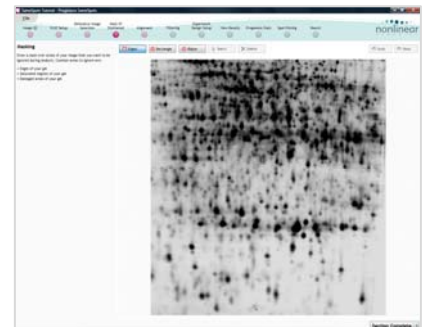
Select a reference image for the other images to be aligned to. You should select one which is a clear, representative spot pattern and has minimal distortion.

Please note - If you are analysing your own images you will next be required to complete the Image Licensing stage by entering your license code in the box. If you are analysing the tutorial images this is not required.



Mask of Disinterest

Here you can exclude areas of your image from analysis. The image you see is the selected reference image, which all of the images in the experiment are aligned to. Any excluded areas will have no spot detection performed during analysis. Areas you may wish to exclude are edges of your image and saturated areas of your image.



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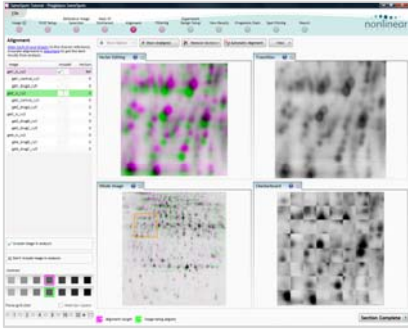
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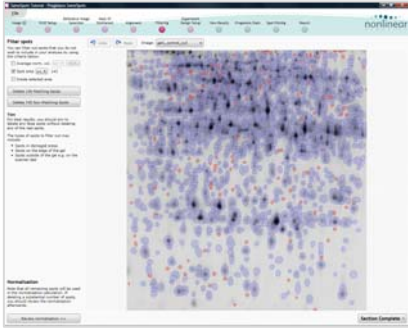
View Results

Alignment is the most important stage in the SameSpots workflow. It corrects positional variation between images, ensures 100% matched spots and enables robust statistical analysis. In a DIGE experiment, each Internal Standard image is aligned to the reference, and the Cy3 / Cy5 is aligned to their Internal Standard. A good first approach is to add alignment vectors automatically, then check the alignment using the visual tools and make any corrections by adding manual vectors. View the images unaligned to add manual vectors and see the affect by switching to an aligned view.



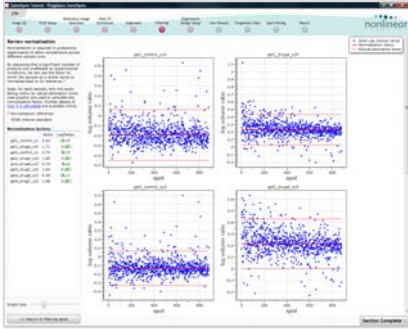
Filtering

This stage allows you to filter out any spots that you don't want to include in the analysis. There are several criteria to select from, and a quick and easy way to remove spots is to right click on the largest spot you want to remove, and choose to use its area or normalised volume to set a filter. The filter is applied to all the images in the experiment.



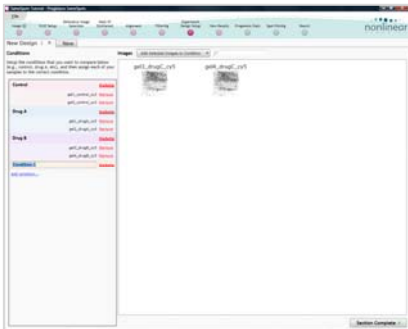
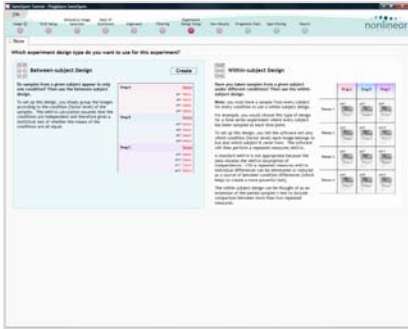
Review Normalisation

Click the "Review Normalisation" button within the Filtering stage and you can visualise the results of normalisation on a scatter plot. You can also see the normalisation factor for each image. If you filter out a large number of spots, you can view the effects this has on normalisation.



Experiment Design Setup

Here you arrange your images into groups for all the comparisons you wish to make. You can choose to set up "between-subject" experiment designs and "within-subject" experiment designs (e.g. timecourse experiments). For this example set up a "between-subject" design and group the images by treatment.



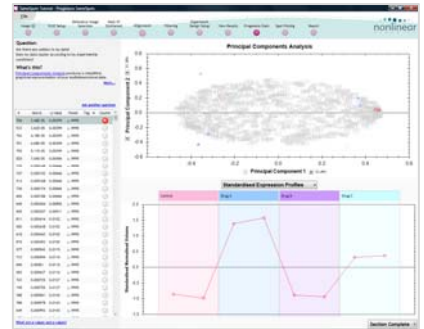
View Results

This stage shows a list of spots which are sorted by Anova (p-value). This helps you determine which spots are displaying interesting changes that you may wish to explore further. By scrolling through the list, you can view the spot expression profile, 2D / 3D montage view and see where the spot is located on the image. Spot editing can also be performed at this stage, which only needs to be done on a single image as any changes are propagated across all images. All spots are selected to be viewed in the next stage which is Progenesis Stats.



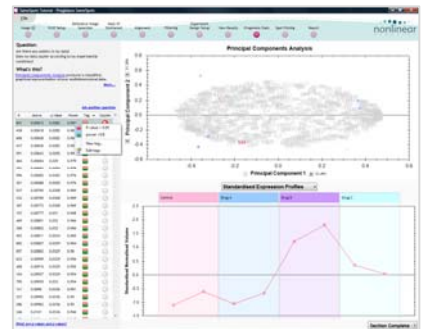
Progenesis Stats

In Progenesis Stats you will first see the PCA (Principal Components Analysis) view which allows you to check that your images are clustered together as per their experimental groups. If one of the images was an obvious outlier, you would want to investigate this further. PCA is an unsupervised technique (i.e. it does not use any knowledge of the experiment groupings), each colour coded ball is an image, and by hovering over it you can see the file name of this image.



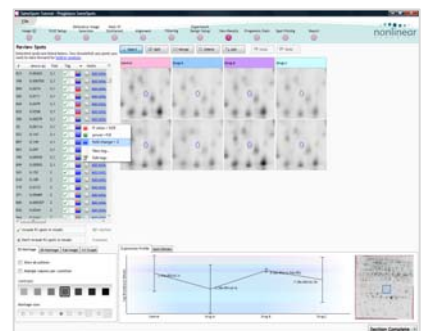
Progenesis Stats

We suggest that you narrow down the interesting spots which you want to pick for mass spec analysis. To do this you can use tags to group together spots with the same characteristics. Spots can be tagged by highlighting a group of spots and right clicking to label them with a coloured tag. You may want to tag spots according to their expression profile (using the correlation analysis), by p-value, q-value, power or fold change.



View Results

Tags can be added in various places in the analysis - you can move between the steps using the workflow along the top of the program. You select which tagged spots to view from the tag drop down.



In this example we suggest tagging spots with p-value < 0.05 and power > 0.8 and filtering the view to show only spots with these characteristics.

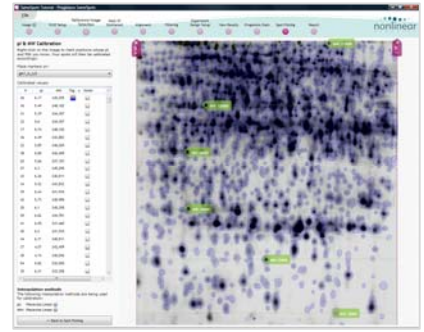
You can then "Ask another question" of your data and group proteins according to their expression profiles by doing a correlation analysis. The dendrogram is interactive so you can click, select and zoom on different areas. You can also drag the grey sliding bar on the dendrogram to highlight the different expression groups which are colour coded.



Groups of spots can be highlighted on the dendrogram and tagged so you can refer to their expression profile alongside the other characteristics you have focussed on.

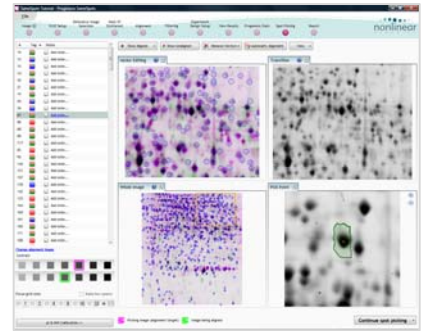
pl & MW Calibration

You can quickly calibrate spots by right clicking and adding a pl or MW value to either known spots or a molecular weight ladder. Calibration values are updates on the fly as marker points are added and are displayed alongside all the spot data in View Results.



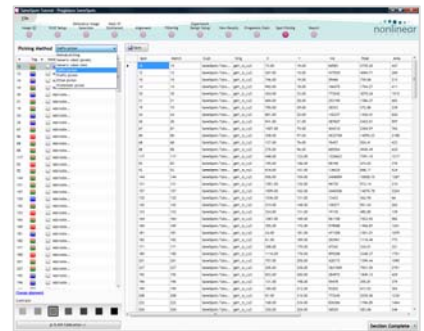
Spot Picking

The first step is to select the image of the gel you wish to pick from. An experimental image then needs to be aligned to this image using the same approach as the earlier "Alignment" stage. Often the images can be quite distorted so more work may be required to perfect the alignment. You can view the spots you have selected / tagged for picking at this stage and adjust the picking point if required on the Pick Point view.



Spot Picking

You get final confirmation of the spots you are about to pick and they are annotated on the whole image view. You are then able to select your picking method and save the spot details in the appropriate format.



Report

In the final stage you can create a customisable report which summarises your results and provides an easy means to share results with your colleagues.

