

Product Specification:

Progenesis™ SameSpots v4.5

SpotCheck Gel Quality Control

- Quantitative quality control of 2D gel images
- Analyse images which will form the gold standard using the SameSpots workflow
- Convert the analysed experiment into a gold standard
- Specify test image pass criteria:
 - Standard deviation limit
 - Percentage of spots to be within the standard deviation limit
- Compare test gels to gold standard
- Straightforward pass or fail result
- Colour codes spots by standard deviations on whole image view
- Data table showing a range of measurements for test gel and gold standard
- Option to export all spot measurements as .csv file
- Generate PDF report of test image result

Image Quality Control and Experiment Set Up Specifications

Experiment set up

- Analysis of single stain and DIGE experiments
- Supports multiple recommended image formats
 - .tiff
 - .img
 - .png
 - .gel
 - .mel
- Create new experiment using alignment reference and spot outlines from previous experiment
- Search for an experiment in your list
- Map protein IDs from previous experiment onto new experiment which has used the same alignment reference
- Add images from multiple folders
- Ability to add additional gel images to an analysed experiment

Clip Gallery

- Save images and tables across the analysis workflow to the clip gallery
- Images saved as high resolution (300dpi) .png fil

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- Enter a title and description for each clipping saved
- Option to export all clippings and notes

Image quality control

- Loads and examines a wide range of image formats
- Unlimited number of images in an experiment
- Automatically examines images using the following QC checks:
 - Image format
 - Image compression
 - Level of saturation
 - Greyscale
 - Dynamic range
 - Intensity bit depth level
 - % intensity level
 - Previous image editing
- Provides feedback and recommendations following QC check
- Image intensity histogram provides feedback on intensity levels in use and dynamic range
- Highlights images which have not passed the quality control process and reports on all reasons for failure
- Tick / cross buttons to individually include / exclude images from further analysis
- Image manipulation tools
 - Rotate
 - Flip
 - Invert
 - Crop

Mask of disinterest

- Ability to draw a mask of disinterest which is excluded from the analysis
 - Exclude area outside rectangle
 - Exclude area within rectangle
 - Exclude area within an ellipse
- Ability to edit a mask of disinterest

Image Alignment Specifications

- Enables a near pixel level correction of image distortion through the addition of alignment vectors
- Enables correction of dye related offsets in DIGE image multiplexes
- Automatic or user controlled selection of reference image for alignment
- Automatic placement of alignment vectors
- Assisted placement of manual vectors
- Coordinated views and tools for rapid verification / correction of alignment
- Exported images retain the grey level calibration in .gel and .img file formats

Alignment visualisation

- 4 simple integrated and interactive image views on User Interface (UI)
 - Zoomed alignment view
 - Alpha blend view with auto zoom for accurate vector placement
 - Whole Image overlaid view
 - Zoomed Chequerboard view
- Separate contrast levels can be set for alignment target and image being aligned
- Interactive image views can be expanded to full screen size
- Quick, simple image focus using one-click selection from displayed list

Alignment controls

- Adjustable alignment focus grid size
- Automatic and manual control of positioning the alignment focus
- Systematic mouse/keyboard driven navigation of alignment focus

- Drag and drop placement of alignment vectors
- Show aligned view with option to:
 - Apply alignment change
 - Always show aligned
- Show unaligned to view vector length and orientation
- Flexible options for vector removal
 - Single vector removal by right mouse click
 - Remove automatic vectors in current square
 - Remove automatic vectors from the whole image
 - Remove all vectors in the current square
 - Remove all vectors

Viewing and information options

- Option to change alignment overlay colours
- Display alignment vectors
- Display aligned grid
- Tick / cross buttons to individually include / exclude images from further analysis

Analysis / Review Specifications

Performing SameSpots analysis

- Select which experimental images contribute to the SameSpots outline map before analysis
- Automatic analysis of aligned image set
 - Spot detection using SameSpots method
 - Background subtraction
 - Normalisation
 - Matching of all spots across experiment

Filtering specifications

- Filters out spots from the SameSpots analysis
- Range of selection criteria for filtering
 - Filter by average normalised volume
 - Filter by spot area
 - Filter by a combination of average normalised volume and spot area
- Filter out spots in a selected area of the image
- Right click on selected spot to set average normalised volume limit or spot area limit for filtering
- Undo / redo filtering

Review normalisation

- Plot of each spot, ordered by ascending raw volume, against the log of the volume ratio to show the effect of normalisation
- Graph shows normalisation factor and robust estimation limits to aid assessment
- Separate plot for each image in the experiment
- Calculates the normalisation factor and the log of the normalisation factor for each image with values displayed in data table
- Option not to perform normalisation (non-DIGE experiment only)
- Adjustable graph size

Experiment design set up

- Group images according to experiment structure
- Choice of 2 experiment structures:
 - Between subject (samples from a given subject appear in only one condition)
 - Within subject (samples from a given subject under different conditions e.g. time-course experiment)
- Ability to set up multiple experimental groupings
- Image search facility to assist with group set up in large experiments

- Rename image groups
- Colour coding of image groups
- Ability to add and remove images from image groups
- Ability to delete groups

Review spots of interest

- Automatic identification of interesting spots according to ANOVA p-values
- Spots ordered by ANOVA p-value
- Switch between experimental groupings and all views are automatically updated
- Spot IDs remain consistent when experiment groupings are changed
- Tick / cross buttons to individually include / discount interesting spots and number of included spots displayed
- Click and drag to select multiple spots to be ticked / crossed
- Visible count of number of spots ticked / crossed
- Automatically advance through spot list
- Review Spots data table can be expanded and includes:
 - Notes
 - Max CV (%)
 - Highest Mean
 - Lowest Mean
 - X Position
 - Y Position
 - Area
 - pI
 - MW

Spot tags

- Colour coded tags to assist with data exploration
- Quick Tag marking spots for a user defined:
 - Anova p-value
 - Fold change
- Add name label to spot tag
- Filter data views by tags using the following tag criteria:
 - Spots that have all of the tags specified
 - Spots that have at least one of the tags specified
 - Hide spots that have any of the tags specified
- Tag editing
- Spots can be tagged multiple times

Viewing options

- Zoom navigation view
 - Click and drag of focus area automatically updates the review spots list, 2D montage and 3D montage view
 - Click on spot for zoom view
- 2D montage view
 - Show current spot or all spots
 - Adjustable contrast
 - Adjustable montage view size
- 3D montage view
 - Select images for showing in 3D
 - Show current spots or all spots
 - Click and drag to reposition 3D view
 - Rotate option
 - Adjustable peak scale
 - Contour display option (where supported by graphics card)
- Whole image view
 - Shows positions of selected spots
 - Option to show all spot outlines

- Option to show spot #, notes or accession number
 - Show any areas removed at Mask of Disinterest step
- Graph of maximum Coefficient of Variation (max CV) plotted against spots ordered by:
 - Max CV
 - Spot number
 - Normalised volume

Spot editing tools

- Spot edit performed on a single image propagated across all the images in an experiment
- Editing tools:
 - Split spot
 - Merge spots
 - Delete spot
 - Add spot
- Undo / redo spot editing
- Automatic recalculations and update of measurements table following spot editing
- Single click recalculation of spot order by Anova p-value following editing

Expression profile

- Plot of mean log normalised volume for each experiment group
- Error bars showing 3 standard errors within groups

Spot details table

- Shows the following spot data:
 - Normalised volume
 - Volume
 - Background measurement
 - Peak height

Spot Picking

- Supports a selection of spot picking robots
 - GelPix
 - ProPic
 - Ettan
 - Bruker Proteineer
 - Generic robot (output of spot picking coordinates)
 - Generic robot (output of spot picking location in millimetres)
- Select picking image from within the experiment or browse for separate picking image
- Image manipulation tools for picking image
 - Rotate
 - Flip
 - Invert
- User controlled selection of image which best corresponds to picking gel
- Separate contrast levels can be set for experimental image and picking gel image
- SameSpots pixel level alignment for alignment of experimental image to picking gel
- Manual adjustment of spot pick location
- Zoom control to allow visualisation of spot pick location on whole image view
- Spots selected for picking highlighted on whole image view
- Option to switch off spot labels on whole image view
- Tagged spots shown on whole image view

pI & MW Calibration

- Image to use for calibration selected via drop-down
- pI and MW calibration markers can be added to an image on either known spots or molecular weight ladder
- pI and MW marker position can be adjusted
- pI and MW values in data table updated on the fly

- Piecewise linear interpolation method used for pI and MW calibration
- pI and MW values displayed in Review Spots data table
- pI and MW values exported to picking robot (where supported by the robot's file format)

Import protein information

- View protein import identification in SameSpots data views
- Notes field editable on export and import
- Manages protein data in:
 - .csv format
 - Mascot XML format
- Label spots on full image view with accession number

Reporting

- Customisable experiment report
- Select spots for inclusion in report
- Reference image with selected spots highlighted
 - Option to annotate with spot ID
 - Option to annotate with spot notes
- Experiment design table showing structure of experiment
- Spot table options include:
 - Anova (p-value)
 - Fold change
 - Tag information
 - Spot notes
 - Spot ID
 - Normalised volumes
- Spot details options include:
 - Spot position
 - 2D montage view showing spot outline
 - Option to exclude images from 2D montage view
 - Spot expression profile showing mean log normalised volume for each experiment group and error bars showing 3 standard errors within groups
- Print and save report

Export Spot Measurements

- Option to export all spot measurements as .csv file
- Tick box to select fields to be exported
- Data which is exported can be controlled by tag filters
- Data which is imported is tagged to allow easy identification and linked back to the original spot data

Additional Specifications (using Progenesis PG240)

Spot Measurement

- INCA image filtering and noise reporting with Data Quality Control measurements
- Statistical comparisons using normal and paired t-test
- Error information viewed as:
 - Coefficient of Variation
 - Standard Error of Mean
 - Percentage SEM
- Data displayed in measurements (single gel)
- Data displayed in comparisons (multiple gel)
- Displays multiple spot fields including difference field
- Spot annotation
- Advanced parameters for display options

Normalisation

- Normalise to single or group of spots
- Normalise to spot group average or collective volume

Intensity Calibration

- Map image pixel values to known optical densities
- View calibration curve
- 5 curve-fitting algorithms

Internet Facilities

- Internet querying of Federated databases
- Automatic HTML web page builder