http://www.frogdance.dundee.ac.uk

GelEval 1.37 Manual – Contents

Overview

GelEval is a software tool designed primarily for molecular biologists for quantifying images of electrophoresis gels. GelEval allows you to measure the densities of gel bands and gel lanes. Starting from the scanned image of a gel, GelEval allows you to delimit the boundaries of individual bands and lanes. The total density of individual bands can then be measured. If your gel contains known standards, line-fitting can be applied to express band intensity relative to the standards. Alternatively, lanes can be scanned to give a graphical representation of intensity as you move from top to bottom of the delimited region. All these features are presented with an easy-to-use interface which use intuitive Macintosh actions. All your measurements can be saved to a quantitation file for future reference.

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What GelEval Can and Cannot Do

At its core, GelEval simply measures the density of pixels in an electronic scan of a gel. It is your responsibility to ensure that this electronic density reflects the abundance of your protein or fragment of DNA. You should be aware that any of the steps required to create a signal (recognition of a protein by an antibody, use of a chemiluminescence reagent) may not provide a linear measure of the item you are trying to quantify. GelEval cannot make non-linear signals linear again. Particular care is needed if the signal has been captured on X-ray film after chemiluminescence detection. The response of X-ray film to light is sigmoidal and is only linear in its mid-range - very weak signals tend to disappear, and very strong signals burn out the film. The response to transmitted light - for example if Coomassie-stained gels are illuminated from below - can be even more complex. In order to demonstrate that the response to signal is approximately linear, it is recommended to use some sort of loading standard, for example a serial dilution of the protein of interest. GelEval can then quantify your experimental samples with reference to these standards (see **Setting and Using Standard Values** for more information).

It is recommended that GelEval is used on raw gel images, and not on images that have been processed (for example by Adobe Photoshop or similar). Tampering with the raw image data will compromise the quantitation. GelEval provides a number of different ways of filtering out ‘background’ signal from the gel (see **Defining the Background** for more information). Again it is your responsibility to ensure that the signal that is removed consists only of background, and does not artificially reduce the real signal.

GelEval saves both the original image file and all its quantitation data in a custom file with an extension of ‘gevl’. The original image file can be extracted unchanged from the .gevl file.

GelEval 1.37 assumes that the gel consists of a dark signal on a light background (as would be produced by autoradiography or chemiluminescence). If you have a gel consisting of light bands on a dark background (for example an ethidium-stained DNA gel), use the *Invert Pixels* command before doing any analysis.

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System Requirements

GelEval 1.37 requires Mac OS 10.7 or higher.

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Installing GelEval
Copy the GelEval folder to your Applications folder. Double-click on the GelEval icon, and follow the onscreen instructions.
GelEval may not start up if you have security settings that prevent use of applications downloaded over the internet. GelEval 1.37 is not code-signed, and can only be used if the Macintosh preferences have been set to allow the use of downloaded applications. To do this, open System Preferences... under the Apple menu on the extreme left hand end of the menu bar; then click on the ‘Security & Privacy’ icon on the top line. Under the ‘General’ tab, select the ‘Anywhere’ radio button under the heading ‘Allow applications downloaded from’. In order to access this option, you may have to unlock the settings by clicking on the padlock at the bottom left hand corner.

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File formats
GelEval can read gel images saved in the following formats: tiff, jpeg, gif, png. The bit depths can be either 8-, 16- or 32- bits per pixel. Some unusual tiff formats are currently not supported; if the image does not display correctly, please open the image file in Photoshop or equivalent, and resave in tiff format. The quantitation data can be stored in a custom GelEval file (.gevl format) which also contains all the image data. Image data can be re-extracted from the custom file unchanged and in its original format.

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What’s new in GelEval 1.37
A full list of changes can be found in the ReadMe file.

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The copyright holders and/or other parties provide the programme "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the quality and performance of the programme is with you. Should the programme prove defective, you assume the cost of all necessary servicing, repair or correction.
In no event unless required by applicable law or agreed to in writing will any copyright holder, or any other party who may modify and/or redistribute the programme, be liable to you for damages, including any general, special, incidental or consequential damages arising out of the use or inability to use the programme (including but not limited to loss of data or data being rendered inaccurate or losses sustained by you or third parties or a failure of the programme to operate with any other software), even if such holder or other party has been advised of the possibility of such damages.

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Purchasing GelEval

A lot of work went into developing GelEval, and we believe that this is reflected in its high quality. Please support the developers at FrogDance Software by buying GelEval. This will allow us to continue developing GelEval and motivate us to write new scientific programmes for the Macintosh.

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Registering GelEval

The demo version of GelEval limits you to 20 uses of the programme. If you buy a full version of the programme, you are also entitled to access to technical support (see "**Technical Support**").

The startup screen of the demo version of GelEval has a button marked "Buy GelEval" which takes you to a screen where you can buy GelEval online. You can also access this screen using the *Buy GelEval...* command under the *GelEval* menu. A licence for a single copy of GelEval costs $15, a licence for 5 copies of GelEval costs $30 and a licence for 10 copies of GelEval costs $50 (all prices quoted in U.S. dollars). **Each license permits you to install and run GelEval on only a single computer at any given time.** Purchasing is made through the Kagi online store, which uses VeriSign Digital ID for all secure transactions. For more details see: http://shop.kagi.com/help.

If you buy a single copy of GelEval, the copy you are using changes from the demo version to the full version. You can move the licensed copy of GelEval to another computer by following the instructions in "**Moving the licensed copy of GelEval to another computer**".

If you buy 5 or 10 copies of GelEval, the copy you are using changes from the demo version to the full version. This copy also serves as a master copy from which further copies of the programme can be registered, as explained in "**Installing additional licensed copies**".

If you want to buy a licence for more than 10 copies of GelEval, please contact FrogDance Software at frogdance@lifesci.dundee.ac.uk.

You can view your serial number and registration code using the *Show Registration Details...* command under the *GelEval* menu. Using the *Go To GelEval Website...* command under the *GelEval* menu opens your default browser and takes you to the FrogDance Software website (http://www.frogdance.dundee.ac.uk).

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Moving the licensed copy of GelEval to another computer

When a copy of GelEval is registered, it is licensed for use on only a single computer. If you want to move it to another computer, transfer it as you would for any Macintosh file. When you open it on the new computer, you will be asked whether you want to transfer the licence to the new computer.

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Installing additional licensed copies

If you buy multiple licences of GelEval, the copy of GelEval used for the purchase automatically becomes registered. This copy also serves as a master copy to register additional copies of GelEval on other computers. If you want to move install GelEval on other computers, copy the registered version as you would for any Macintosh file. When you open it on the new computer, you will be asked whether you want to apply the licence to the new computer.

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Technical Support
Technical support is available from FrogDance Software for users of registered copies of GelEval. E-mail us at frogdance@lifesci.dundee.ac.uk and describe the problem as clearly as possible. Please also include in your e-mail address, the version number, serial number and registration code. All these numbers can be found using the *Show Registration Details...* command under the *GelEval* menu.

The *GelEval Manual* command found in the *Help* menu opens this manual from within the program.

Limited technical support is available for unregistered users, but whether or not you are a registered GelEval user, FrogDance Software welcomes user feedback on bugs, ways to improve GelEval and suggestions for new features.

Please also contact us at the above e-mail address if you would like to explore options for purchasing more than 10 licences of GelEval.

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Upgrades
From time to time, upgraded versions of GelEval may be produced. It is our intention to make all future upgrades free for customers who have purchased earlier versions. GelEval 1.37 uses the Sparkle framework to automatically check for updates. If you wish, you can initiate a check by using the *Check For Updates…* command in the *GelEval* menu. Follow the on-screen commands to perform the upgrade. Instead of using the Sparkle upgrader, you can simply download the upgraded version from the GelEval web site. The upgraded version will still know that it is licensed on that computer.

Current version: GelEval 1.37, June 2013.

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Opening and Saving Files

GelEval can be used to analyse image files saved in a variety of formats. All the GelEval information can be saved along with the image in a custom GelEval (.gevl) file. The original image data can be subsequently exported unchanged from the custom GelEval file in its original format.

Opening a File with GelEval

To open an image file with GelEval, use the *Open…* command located in the main *File* menu, or press ⌘O. Recently-used files are listed under *Open Recent* in the *File* menu. GelEval can read files in tiff, jpeg, gif and png format at 8-, 16 or 32- bits per pixel. Some unusual tiff formats are currently not supported; if the image does not display correctly, please open the image file in Photoshop or equivalent, and resave in tiff format. GelEval can also read files in its own custom format, which are indicated by a .gevl extension. Custom GelEval files can be opened either using the *Open…* command in the GelEval programme, or by double-clicking on the file icon in the Finder.

GelEval 1.37 can also open files created by previous versions of GelEval (which have a .gev extension).

Double-clicking on a GelEval file opens it up in the GelEval programme.

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Saving the Quantification Data as a GelEval File

All the quantification data can be saved along with the original image data in a custom GelEval file (with a .gevl extension). Use the *Save As…* command from the *File* menu or press <*shift*>⌘S to converted an image to a GelEval file type. GelEval files can be saved using the standard *Save* or *Save As…* commands from the *File* menu or by pressing ⌘S or <*shift*>⌘S.

GelEval files store the following data:
- the original image in tiff format
- the image rotation
- whether pixel densities are inverted
- the rectangles
- standard values
- the background values
- the notes

If the file was originally an older .gev format (created by previous versions of GelEval), the file will be converted to the new .gevl format. You will be given the option to overwrite the old .gev file, or keep it.

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Exporting the Original Image Data

The original image can be extracted from a GelEval file and saved to disk using the *Extract Original Image File…* command from the *File* menu. You will be asked where to save the file. The image file will be exactly the same as the one you originally opened with GelEval.

You can also get access to the original image file using the Finder. Click on a .gevl file whilst holding down the <ctrl> key. From the contextual menu that appears, select Show Package Contents. This will reveal the contents of the .gevl file: your original image file, plus a file called GelEvalData which contains all the quantification data.

If you copy a .gevl file to a computer which does not have a copy of GelEval installed, .gevl files will appear as standard Macintosh folders, containing the original image file plus the GelEvalData quantification data.

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Exporting the Annotated Image

The annotated image, optionally containing quantification rectangles and text, can be exported in tiff format. You can specify the resolution of the image for export, to prevent the text from becoming excessively pixelated. To export the annotated image, select *Export* > *Export Annotated Image…* from the *File* menu. A Save panel is displayed which allows you to set the name and location of the exported image. After pressing the '*Save*' button, you are presented with a sheet that allows you to select a range of options for the export. You can choose to export text, rectangles and/or rectangle names by checking the individual items. You can also choose to increase (or decrease) the base resolution of the tiff file. Increasing the resolution may be useful to prevent the text annotations from appearing pixelated on the exported image.

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Exporting the Quantification Table

The data from the Quantification Table can be exported as a tab-delimited text file that can be read by most by spreadsheets or graphing programs. To export the data, select *Export* > *Export Quantification Table…* from the *File* menu. A Save panel is displayed which allows you to set the name and location of the exported image.

An alternative way of exporting quantitation data is to copy into onto the pasteborad, where it can be pasted into other applications. When the Quantification Table is foremost, the *Copy* command from the *Edit* menu (or pressing ⌘*C*) copies the numbers in text format, so that they can be pasted into spreadsheets, graphing programs or word processors.

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Exporting Graphs

Graphs can be exported as pdf files. When a graph window is foremost, select *Export* > *Export Graphs…* from the *File* menu. A Save panel is displayed which allows you to set the name and location of the exported graph file. All graphs associated with the current document are saved as a multipage pdf file, with each graph on a separate page.

An alternative way to export graph data is to copy it onto the pasteboard. When a graph window is foremost, the *Copy* command from the *Edit* menu (or pressing ⌘*C*) copies a pdf version of the graph onto the pasteboard, which can then be pasted into a variety of image processing programmes (Adobe Illustrator, Preview etc). At the same time, the coordinates of the graph are also copied onto the pasteboard in text format, for pasting into spreadsheets or graphing programmes.

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Exporting the Notes Document

The Document Notes (see Keeping Notes) can be exported as a text file. Select *Export* > *Export Document Notes…* from the *File* menu. A Save panel is displayed which allows you to set the name and location of the exported text file.

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Rotating, Scaling and Cropping the Image

Changing the Image Scale

There are three ways to see the image at different scales. You can use the magnifying glass tool from the toolbar. This allows you to zoom in and out centred on specific regions of the gel, changing the scale two-fold each time. Clicking once on the magnifying glass tool gives you the zoom in magnifier; click again on the toolbar control and you get the zoom out magnifier. If you hold down the <alt> key whilst the magnifying glass is selected, you will toggle between the zoom in and zoom out tool.

You can also zoom in and out using the zoom commands *Zoom In* and *Zoom Out* which are found in the *Window* menu. These commands can also be invoked with the key combinations ⌘= and ⌘-. Each time you zoom with these commands the scale changes two-fold, with the centre of the image staying fixed.

Alternatively you can set the scale directly using the scale box in the lower left hand corner of the image screen. Enter the desired scale (expressed in %), then press <*return*>. A scale of 100% means that each pixel of the image is displayed as exactly one pixel on the screen.

If you activate the *Scale Image To Window* command from the *Window* menu, the entire gel is scaled to the current window size.

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Rotating the Image

The image can be rotated by using the rotation commands found in the *Window* menu. The *Rotate Image Clockwise…* and *Rotate Image Anti-Clockwise…* commands each bring up a screen allowing you to enter the number of degrees by which the image will be rotated. Pressing the *OK* button starts the rotation process, which may take several seconds. Pressing the *Cancel* button dismisses the screen without rotating the image. Note that quantitation rectangles are not rotated with the underlying image. Also note that any crop that has been applied to the image will be lost if the image is rotated.

The *Revert to Unrotated Image* command resets the rotation back to the value of the original image file as it was when first opened by the GelEval programme.

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Cropping the Image

A temporary crop can be applied to the image so that extra space around the area of interest is eliminated from the view. To crop an image, first adjust the visible portion of the gel using the scroll bars and the window's resize handle (the square at the extreme bottom right of the window) so that the window displays only the portion of the image you want to see. Then select *Crop Image To Window* from the *Window* menu. This temporarily removes all areas of the image that are not currently visible. You can restore the full extent of the scanned image by selecting *Revert To Uncropped Image* from the *Window* menu.

To prevent degradation of image quality, crops are not maintained if the gel image is rotated.

Inverting Pixels

The *Invert Pixels* command found in the *Window* menu inverts the representation of the image, turning a black-on-white image to a white-on-black image and vice versa.

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Quantitation Rectangles

Areas of the image can be delimited by quantitation rectangles. These rectangles have a variety of uses. They can enclose a single area, such as a band on a gel, to measure the density of the band. Other rectangles can be used to define the background density. Another way of using rectangles is to outline an entire lane on a gel. The density of the lane can be scanned vertically and then plotted out in a graph.

Creating Quantitation Rectangles

Areas of the gel to be quantified are delimited by quantitation rectangles. Rectangles are created with the mouse in Rectangle Creation Mode.

There are three ways of setting Rectangle Creation Mode:
1) Select the crosshair cursor from the segmented button at the top of the image window.
2) Select *Rectangle Creation Mode* from the *Rectangles* menu; or
3) Press ⌘*R*.
When over the image, the mouse changes to a crosshair to show that you are in Rectangle Creation Mode.

Once in Rectangle Creation Mode, quantitation rectangles are created by clicking on the image where you want to rectangle to start, dragging to where you want the rectangle to end, and then releasing the mouse button.

Quantitation rectangles can also be created by using Copy and Paste commands (see '**Copy/Cut/Paste commands**').

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Selecting Quantitation Rectangles

In order to manipulate quantitation rectangles, the mouse must be in Rectangle Selection Mode.

There are three ways of setting Rectangle Selection Mode:
1) Select the arrow cursor from the segmented button at the top of the image window; or
2) Select *Rectangle Selection Mode* from the *Rectangles* menu; or
3) Press ⌘*E*.
When over the image, the mouse changes to an arrow to show that you are in Rectangle Selection Mode.

To directly select a quantitation rectangle, simply click somewhere on its outline. 'Handles' (small squares) appear at the rectangle's corners and edges to show that the rectangle is selected.

To select several quantitation rectangles, hold down the <*shift*> key whilst selecting.

To select groups of rectangles, click on the image where there is no quantitation rectangle and drag the mouse. This creates a selection marquee shown by a dashed line. All rectangles that intersect the marquee are selected when the mouse button is released.

All the quantitation rectangles can be selected by using the *Select All* command from the *Edit* menu, or pressing ⌘*A*.

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Changing a Quantitation Rectangle's Name
When a quantitation rectangle is first created, its name, shown just above the rectangle, is set to 'rect' plus a number. You can change the name by selecting the rectangle (see '**Selecting quantitation rectangles**'), and then placing the cursor over the name. The cursor turns to an I-beam to show that name editing is now permitted. Click somewhere in the name, and a blinking insertion point is created. You can then delete or add text as you would with any standard text editor.

A rectangle's name can also be changed using the *Rectangle Specifications* window (see '**Viewing and Editing Rectangle Specifications'**).

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Changing a Quantitation Rectangle's Size
To change the size of a quantitation rectangle, first select it (see '**Selecting quantitation rectangles**'), so that the handles appear at its corners and edges. The position of a corner can be changed by clicking in the appropriate corner handle and dragging it to the desired position. The edge of a rectangle can be changed by clicking in the appropriate edge handle and dragging it to the desired position. If more than one rectangle is selected and the <*shift*> key is held down when these actions are performed, all the selected rectangles will be resized in the same way.

A rectangle's size can also be changed using the *Rectangle Specifications* window (see '**Viewing and Editing Rectangle Specifications'**).

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Repositioning Quantitation Rectangles
To change the position of a quantitation rectangle, first select it (see '**Selecting quantitation rectangles**'), so that the handles appear in its corners and edges. Click on the rectangle outline again anywhere away from the handles, and drag the rectangle to its desired position. If more than one rectangle is selected and the <*shift*> key is held down when this is done, all the selected rectangles will be repositioned in the same way.

Quantitation rectangles can also by repositioned using the arrow keys. The arrow keys nudge all selected rectangles by one pixel in the direction indicated by the arrow. If the scale of the image is less than 100% each nudge moves the rectangle(s) by one pixel on screen, which may represent more than one pixel in the image data. When the scale of the image is 100% or more, each nudge moves the rectangle(s) by one pixel in the image data.

The position of a group of rectangles can also be changed by aligning or distributing them relative to one another (see '**Aligning and Distributing Rectangles'**) or by using the *Rectangle Specifications* window (see '**Viewing and Editing Rectangle Specifications'**).

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Aligning and Distributing Rectangles
When 2 or more quantitation rectangles are selected they can either be aligned to one another or can be equally distributed relative to one another. Alignment and distribution can be performed relative to either side of the rectangle or its centre. Commands for aligning and distributing rectangles are found in the *Align Vertically* and *Align Horizontally* submenus of the *Rectangles* menu.

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Viewing and Editing Rectangle Specifications
The Rectangle Specifications window shows data for the currently selected rectangle. The Rectangle Specifications window can be activated by double-clicking on a rectangle or by using the *Rectangle Specifications…* command in the *Rectangles* menu.

The Rectangle Specifications window contains three sections:
1) 'Previous' and 'Next' buttons which allow you to cycle through all the rectangles.
2) The rectangle attributes, including name, dimensions, index and standard value.
3) The size of the image data in pixels for reference. This data cannot be edited.
4) A graph of the density of the rectangle as it is scanned from the bottom to the top.

Pressing the 'Previous' and 'Next' buttons allows you to cycle through all the quantitation rectangles in turn.

The rectangle attributes can all be edited:
- The name is what appears above the rectangle on the image window (see '**Changing a Quantitation Rectangle's Name**').
- The Index number defines the order in which the rectangles are stacked on the image. Rectangles that lie above other rectangles have higher index numbers; the bottom rectangle has an index number of zero. The index number determines: a) the order in which rectangles appear in the Quantitation window; b) the order in which density scan graphs are stacked; and c) which rectangle is selected when two overlapping rectangles are clicked with the mouse in Rectangle Selection Mode. The index can also be changed by dragging the relevant rows in the Quantitation window (see '**The Quantification Table**').
- The Standard value is set if the rectangle encloses a band that represents a defined quantity (for example a protein or DNA standard) against which you want to quantify other bands (see '**Setting and Using Standard Values**'). If this field is left blank, no Standard value is set.
- The positions of the four edges of the rectangles can be edited relative to the bottom left hand corner of the image. Two simple limitations apply: 1) the edges cannot lie outside the borders of the image, and 2) the position of the bottom edge must be less than that of the top edge and the position of the left edge must be less than that of the right edge. If these values are changed, the density graph is continuously updated.

The graph shows the Y coordinates of the rectangle relative to the bottom of the image, matching the Top and Bottom coordinates shown under Rectangle Attributes. If you change the size or position of a rectangle with the mouse, or by typing in Top, Bottom, Left or Right coordinates, the density graph is continuously updated. The Rectangle Specifications window can therefore be used to precisely position quantitation rectangles relative to the underlying image density.

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Copy/Cut/Paste commands
The *Copy* command from the *Edit* menu (or pressing ⌘*C*) copies all selected quantitation rectangles onto the pasteboard. The *Cut* command (⌘*X*) also copies all selected quantitation rectangles onto the pasteboard but deletes the rectangles in doing so. The *Paste* command from the *Edit* menu (or pressing ⌘*V*) pastes the rectangles on the pasteboard onto the image at a position offset from the copied rectangle. Their name has an incrementing number appended to it.

A quick way of positioning many copies of a rectangle is to paste them a specific places on the image in Paste At Point mode. Paste At Point mode can be entered when one or more rectangles have been copied onto the pasteboard. There are then three ways of setting Paste At Point Mode:

1) Select the paste-at-point cursor (a square with a filled-in corner) from the segmented button at the top of the image window; or
2) Select *Paste At Point Mode* from the *Rectangles* menu; or
3) Press ⌘*T*.

In Paste At Point mode the mouse changes to a small rectangle with a filled-in left hand corner. If you now click on the image, a rectangle will be pasted with its left hand corner at the position of cursor's black square.

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Deleting Quantitation Rectangles

Selected quantitation rectangles can be deleted by pressing the backspace key or by using the *Delete* command or *Cut* (⌘*X*) commands from the *Edit* menu.

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Vertical Scans

The *Perform Vertical Scans* command in the *Quantification* menu generates graphs of the changing density at different heights in all selected quantitation rectangles. This command adds up the total density in each horizontal row of pixels, optionally subtracts the background and plots the result in a graph. Typically each quantitation rectangle would outline a single lane of a gel, and each graph then reflects the density of a lane at different distances from the top of the rectangle. All the graphs are collected together in a cascading series of windows that automatically repositions itself to prevent the computer screen becoming cluttered.

Performing Vertical Scans

To perform vertical scans, select the *Perform Vertical Scans* command in the *Quantification* menu. If more than one quantitation rectangle is selected when the *Perform Vertical Scans* command is given, each selected rectangle is plotted in its own graph, with the vertical axis of each scan set to give the same maximum value.

The *Show Background Scan* command from the *Quantification* menu plots a graph of the background from top to bottom of the image (see '**Area and Gradient Background**').

There are a number of options that determine how the vertical scan graphs are plotted. These options are all set from the *Quantification* menu.

- *Vertical Scan Display Mode* determines whether the mean pixel density or the total pixel density across the width of the rectangle is plotted.
- *Vertical Scan Background Display* determines whether the background density is subtracted before plotting the graph. If the background is not subtracted, it is plotted in dotted lines on the graph.
- *Vertical Scan Direction* determines whether the scan is performed top-to-bottom (ie top of rectangle at low x value) or bottom-to-top (ie bottom of rectangle at low x value).

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Vertical Scan Graphs

To change either the X or Y axis, double-click on the axis: a response sheet then appears, inviting you to change the maximum axis value (at present it is not possible to change the origin of the graph, which is fixed at 0,0).

When a graph window at the front is highlighted, its graph can be copied onto the pasteboard (using *Edit* > *Copy* or ⌘*C*) for pasting into other applications. Alternatively, when a graph window at the front is highlighted, it can be exported as a pdf file (see **Exporting Quantification Data** for more information) or printed (using *File* > *Print...* or ⌘*P*). If the Page Setup is changed when a graph window is highlighted (using *File* > *Page Setup...* or <*shift*>-⌘*P*), the Page Setup is applied to all the graphs associated with that gel.

To make it easy to read graph values, *Show Live Graph Coordinates* can be activated from the *Quantification* menu. When *Show Live Graph Coordinates* is active and the cursor is over a graph, the graph coordinates automatically appear next to the cursor. It may be necessary to click on the graph to ensure it is the front-most window in the application for the coordinates to appear.

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Graph Cascading

The graph windows are automatically positioned on the screen (cascaded) to make them easier to manage. The entire stack of vertical scans can be moved by dragging one of its component windows with the command key (⌘) held down. An individual graph can be

removed from the cascader by dragging it away from the other windows with the option(<*alt*>) key held down.

If you want to position all the graph windows yourself, you can inactivate automatic positioning by using the *Turn Graph Repositioning Off* command from the *Quantification* menu. The *Turn Graph Repositioning On* command from the *Quantification* menu turns this feature back on.

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Changing Graph Axes
When groups of rectangles are scanned at the same time, the maximum value of the density on the y axis of the each graph is set to the same value, making all the graphs comparable. The maximum value of both x and y axes can be set manually by double-clicking on the axis. This activates a sheet where the maximum value on the axis can be entered.

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Live Graph Coordinates
Numerical values for the x and y values can be displayed for graphs you have created. To turn this feature on, select Show Live Graph Coordinates from the Quantification menu; to turn it off, select Hide Live Graph Coordinates from the Quantification menu. Whenever this feature is active and a graph window is at the front of the application, the x-y coordinates of the position of the mouse are shown next to the mouse when it lies over the plot area of the graph.

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Defining the Background
No gel has a perfectly clear background. Sometimes the background is fairly constant across the gel, but in more complicated cases the background may change as you move up or down a gel. GelEval has three different ways of setting the background to deal with common background problems. By viewing the **rectangle specifications screen** whilst different backgrounds are set, you can get a clear idea of how the different background types affect the way the lane density is calculated.

Area, Gradient and Local Backgrounds
The background density can be set in four different ways: area, gradient or local backgrounds, or no background subtraction.

Area background is defined by the average density of pixels enclosed by one or more quantitation rectangles that you define. This average value is subtracted from all pixels measured in quantitation operations (though the density is never allowed to become negative). See '**Setting an Area Background**' for how to set an area background.

Gradient background is used if the background varies from top to bottom of the image. When a gradient background is set, a separate background density is established for each row of pixels in the image. This value is subtracted from all pixels in the corresponding rows when measured in quantitation operations (though the density is never allowed to become negative). The background gradient is set by selecting rectangles enclosing different regions of the image that reflect the changing background. See '**Setting a Gradient Background**' for how to set a gradient background.

Local background can be used if different lanes have different backgrounds. It uses the top and bottom of a rectangle to determine a different local background for each rectangle. The background density is defined by a straight line between the density of the top of the rectangle to the density at the bottom of the rectangle. As with the other background types, the density is never allowed to become negative. See '**Setting a Local Background**' for how to set a local background.

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Setting No Background Subtraction
If No Background Subtraction is set, the unmodified densities of the gel are used for calculation and graphs. Set this mode by selecting the menu command *Quantification* > *Set Background* > *No Background Subtraction*, or pressing ⌘M.

Setting an Area Background
Area background is defined by the average density of pixels enclosed by one or more quantitation rectangles. This average value is subtracted from all pixels measured in quantitation operations.

To set an area background, select one or more quantitation rectangles that enclose areas of background density. Then use the menu command *Quantification* > *Set Background* > *Set Area Background*, or press ⌘B. If no quantitation rectangle is selected when this is done, the background is set to zero.

The area background can also be entered manually in the *Background Specifications* window. The *Background Specifications* window can be viewed by using the *Background Specifications…* command in the *Quantification* menu. Radio buttons then let you toggle between using area, gradient or local backgrounds (see '**Area, Gradient and Local Background**' for the difference between these). The value of the area background is shown in a text box and can be edited. A value of zero means that the background is completely white; a value of one means that the background is completely black.

The background can also be viewed as a vertical scan; see '**Viewing the Background**' for details.

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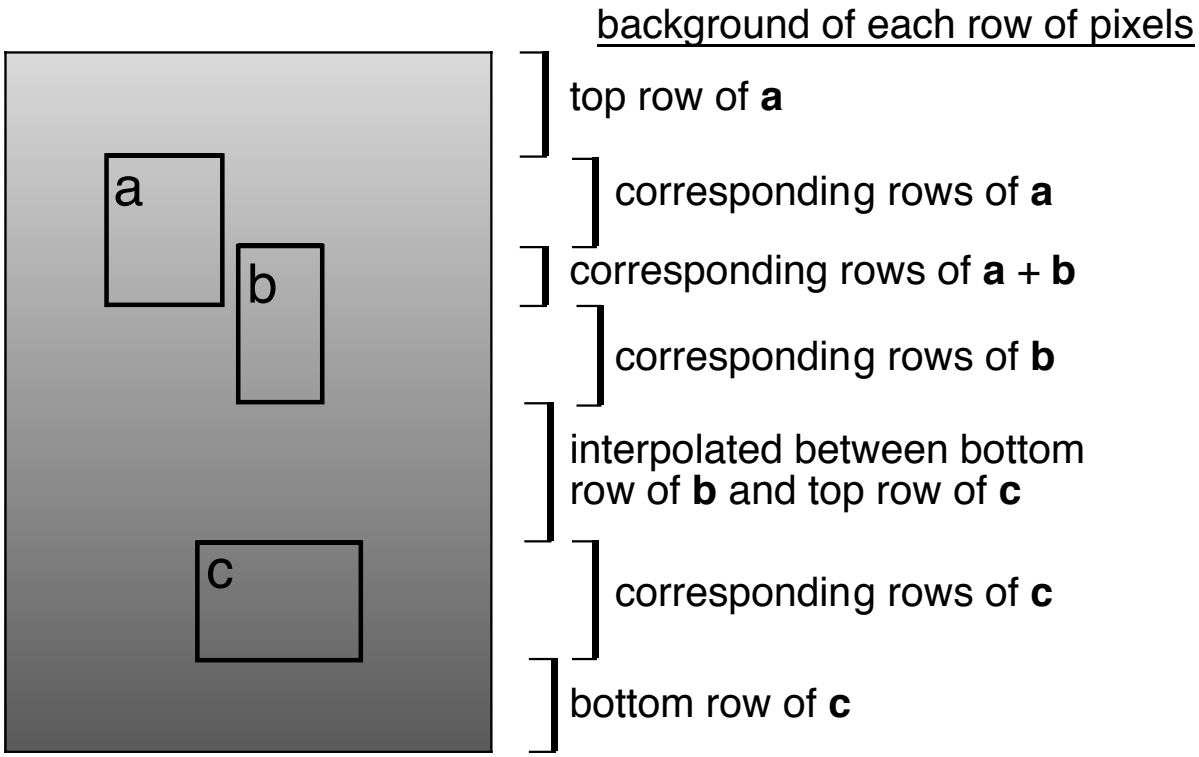
Setting a Gradient Background
Vertical background is used if the background varies from top to bottom of the image. When a vertical background is set, a separate background density is established for each row of pixels in the image. These values are subtracted from all pixels in the corresponding rows when measurements are made.

To set a gradient background, select one or more quantitation rectangles whose vertical extents cover as much of the image background as possible. Then use the menu command *Quantification* > *Set Background* > *Set Gradient Background*, or press ⌘G. The density of each row of pixels in the image is then calculated in the following manner:
1. For each row of pixels contained in one or more of the selected rectangles, the background is set to the average pixel density of the row of pixels in the rectangles.
2. For rows of pixels that do not intersect any of the selected rectangles, but which lie between selected rectangles, a linear interpolation is made between the lowest row of the upper selected rectangle and the highest row of the lower selected rectangle.
3. For rows of pixels that do not intersect with any of the selected rectangles and are either above or below all the selected rectangles, the background is set to the value of the nearest selected row.

An example is given below, showing how the density of each row of pixels is determined when three quantitation rectangles **a**, **b** and **c** are used to define a gradient background.



The background can be viewed as a vertical scan; see '**Viewing the Background**' for details.

If a gradient background has previously been set, but an area background is currently in use (see '**Area, Gradient and Local Background**' for the difference between these), the gradient background can be restored in the *Background Specifications* window. The *Background Specifications* window can be viewed by using the *Background Specifications…* command in the *Quantification* menu. Radio buttons then let you toggle between area, gradient or local backgrounds.

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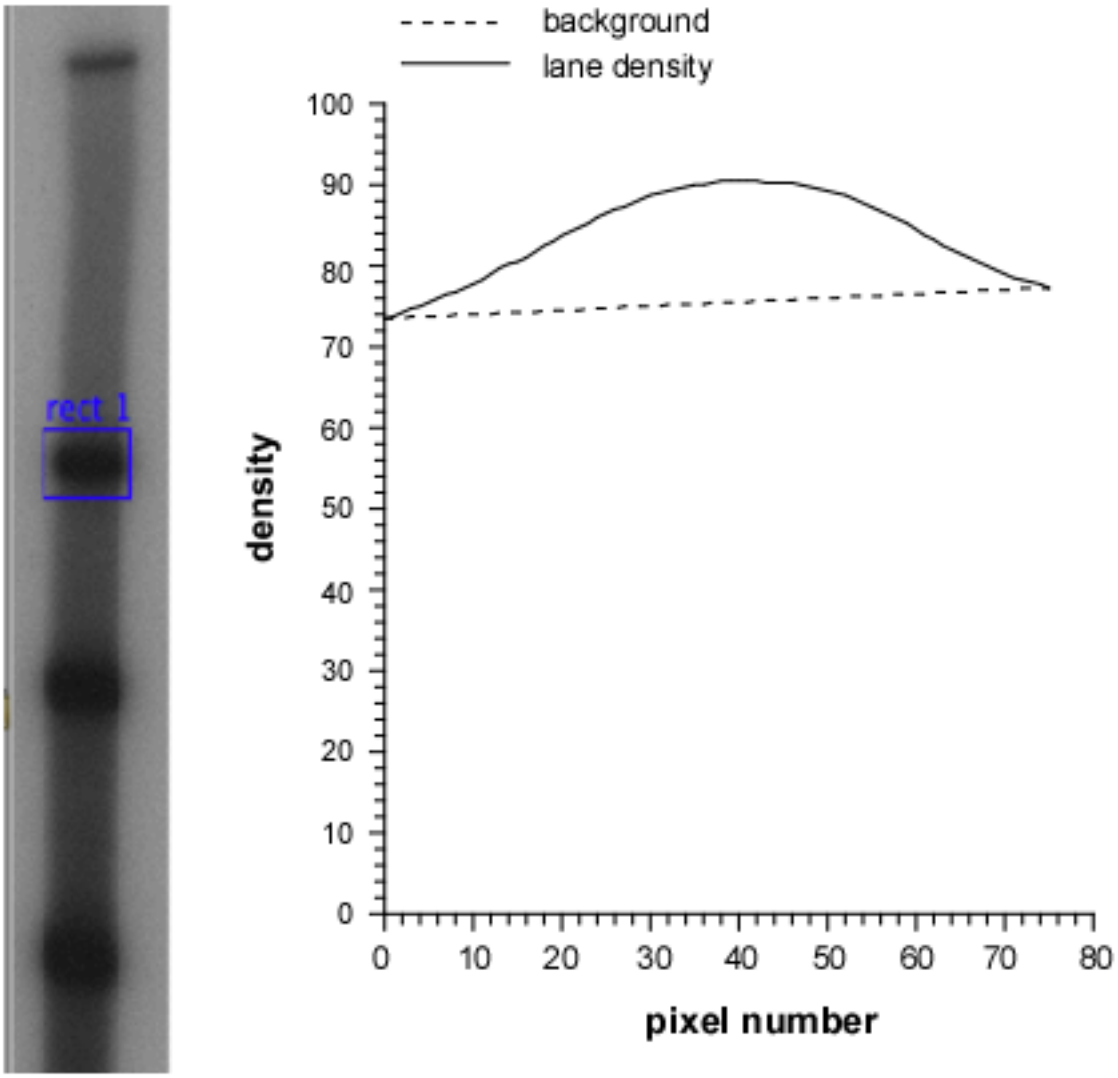
Setting a Local Background

Vertical background is particularly useful when gel lanes are smeary, with the density of the smear varying at different positions down the lane. Unlike the **gradient background**, which applies the same background calculation to all pixels at the same vertical height on the gel, the local background varies from rectangle to rectangle, even if they are at the same vertical height. The vertical background is calculated by finding the mean density of the top row of pixels of a rectangle and the mean density of the bottom row of pixels in the gel, and drawing a straight line between them. Only densities in excess of this line are considered as contributing to the density of the band. An example is given below:



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Viewing the Background

When area or gradient backgrounds are set, the *Show Background Scan* command from the *Quantification* menu plots a graph of the background from top to bottom of the image (see '**Area and Gradient Background**'). The background can also be viewed from the rectangle specifications screen (see **Viewing and Editing Rectangle Specifications**). This has the advantage that when background parameters are changed, or when the rectangle is moved or resized, the scan is automatically updated.

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Quantification

GelEval offers a number of different ways to directly quantify the density of gel bands. These methods make it easy for you to compare the quantity of material present in different bands. Most of the quantitation tools are available in the Quantification Table.

The Quantification Table

The Quantification Table shows information about the density of the image within all the rectangles. To show the Quantification Table, use the *Show Quantification Table* command from the *Quantification* menu or press ⌘U. Each of the rectangles are listed, one per row. The order that the rectangles are listed can be changed by selecting one or more rows and dragging them to a new position. Alternatively, rectangles can be reordered by changing the index number in the Rectangle Specifications window (see '**Viewing and Editing Rectangle Specifications**').

Rectangle name: the rectangle name.
Area: the area enclosed by the rectangle, expressed as a number of pixels.
Density: the total density of all the pixels in the rectangle (0=white, 1=black for each pixel).
Density - bk: the total density of all the pixels after the background has been subtracted from all of them.
Mean density: the mean total density of pixels in the rectangle (i.e. Density / Area). Zero is completely white; one is completely black.
Mean density - bk: the mean total density of pixels in the rectangle after the background has been subtracted (i.e. Density - bk / Area). Zero is completely white; one is completely black.
Standards: a user-supplied number for the value of the density of the rectangle. See '**Setting and Using Standard Values**'.
Line fit value: a value for the density of the rectangle, derived by fitting a straight line through the user-supplied Standards. See '**Setting and Using Standard Values**'.
Interpolated value: a value for the density of the rectangle, derived by drawing straight lines between each of the user-supplied Standards. See '**Setting and Using Standard Values**'.

These values are continuously updated as parameters are changed. Rows can be selected by clicking on them, and then values can be copied onto the clipboard by using the *Copy* command from the *File* menu or pressing ⌘C, ready for pasting into other applications. To select multiple rows for copying, click once in the top row, hold down the <*shift*> key and then click in the bottom row.

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Setting and Using Standard Values

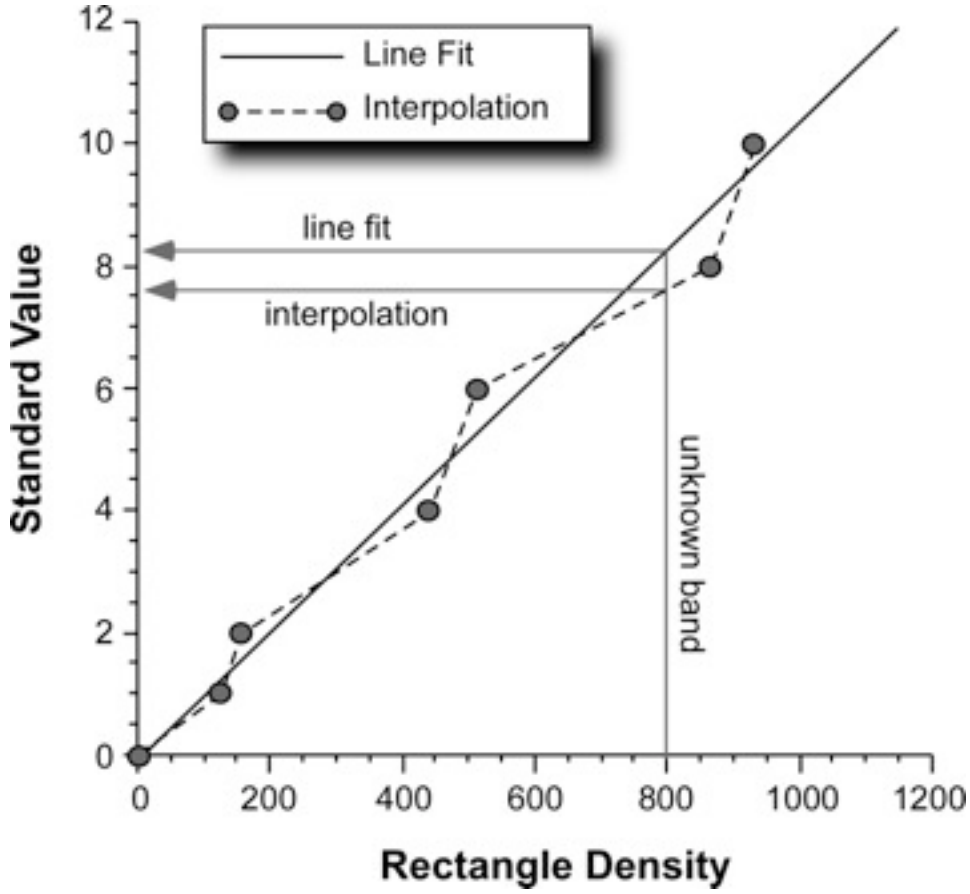
Very often a gel will contain one or more standards - samples that represent known quantities or serial dilutions of a protein or DNA fragment. If the quantity of these standards is entered into the Quantification Table, GelEval can estimate the value of unknown bands. To do this, open up the Quantification Table by using the *Show Quantification Table* command from the *Quantification* menu or pressing ⌘U. For details of all the information listed in this window, see '**The Quantification Table**'. The value of any known standard can be directly entered into the Standards column for the relevant rectangle. The units of measurement (eg mg/ml or µg) cannot be specified, and must be the same for all the standards. GelEval uses two different methods to estimate the density values of unknown rectangles: line fitting and linear interpolation.

For line fitting, GelEval subtracts the background from the density of the corresponding rectangles, and calculates a straight line to fit all of them using the least squares method. A standard value of zero for the background density is automatically included whilst fitting the line. An estimate of the quality of the standard line can be obtained from the correlation

coefficient shown in the Quantification Table (a value of 1 represents a perfect fit). The value for unknown rectangles can then be read from the 'Line fit value' column.

For line fitting, GelEval draws a straight line between the densities of adjacent standard values (having first subtracted the background). A standard value of zero for the background density is automatically included whilst fitting the line. It then reads off the values of unknown rectangles and puts these into the 'Interpolated value' column. Linear interpolation can only be performed on rectangles whose density lies between those of standard values.

The figure below compares line fitting and interpolation methods. Standard samples with values of 1, 2, 4, 6, 8 and 10 units have been used. An unknown band in a rectangle with a density of 800 is estimated as having a value of 7.6 units by linear interpolation and a value of 8.2 units by line fitting.



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Showing the Line Fit Graph

Once the data for standard values has been loaded by showing the Quantification Table (see '**The Quantification Table**') and two or more standard values have been set (see '**Setting and Using Standard Values**'), linear interpolation and line fitting can be used to calculate the intensity of unknown bands. A graph showing the linear interpolation and line fitting used for these calculations can be displayed by using the *Show Line Fit Graph* command from the *Quantification* menu. The X axis gives standard values in user-defined units, and the Y axis gives rectangle densities as measured by the programme. Interpolation between the data points is shown by a dashed line, whilst the line fit is shown by a solid line, just as in the graph above.

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Live Image Density
If you want an easy way to examine the pixel density of different parts of the image, open use the *Show Live Image Density Window* command in the *Quantification* menu. This brings up a small window that floats in from of the image, and continuously reports the density of the pixel directly underneath the mouse, as well as any background value that is applied to that pixel.

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Text

It is important that gels are properly annotated. GelEval allows you to add text to an image file, for example to label individual gel lanes or to identify specific gel bands. Font and style attributes can be customised and the text can be rotated. In addition to adding text to the image, there is also a Notes window, where you can record metadata about the gel, such as for example details of the experimental protocol (see **Keeping Notes** for more details).

Creating a Text Object
Text Objects are created using the text creation tool - the 5th item on the segmented control at the top of the window, denoted with a letter 'A'. After the text creation tool has been activated, the cursor turns into the typical text I-beam cursor. Click the I-beam cursor at any point in the image to create a text object at that position. You can now type text onto that place in the image (or paste text from the clipboard). Once you have finished adding text at that point, click anywhere away from the current text object, and editing will be ended.

When a text object is first created, typing occurs on a single line, with the text object expanding horizontally with the text. Once this initial typing session is over, you can change the width of the text, by dragging the handles (see **Resizing Text Objects)**; the height of the text is then automatically adjusted to encompass all the text.

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Editing Text Objects
All the features of the text in a text object are editable using the standard Macintosh commands found in the *Text* menu. To begin an editing session, click on the text object you want to edit (you don't have to be in text editing mode to do this)- this selects the text object for editing (its boundary is shown by a blue rectangle and resize handles appear). When a text object is selected and the cursor is positioned over the text, the cursor turns into a text-editing I-beam cursor. Click the I-beam cursor on the place in the text where you want to start editing. If the text is rotated, the text temporarily becomes unrotated and the text editing point is set at the end of the text (unfortunately, the Macintosh text editing system cannot edit text while it is rotated). You can then add or delete text as normal using the keyboard. In addition, the text style attributes can be changed using the commands in the *Text* menu. If a substring of the text is highlighted (by dragging the I-beam cursor over it), the style changes will apply only to the highlighted text. Style attributes that can be changed are:

Justification: sets the style to Align Left, Align Right, Centre of full Justification.
Fonts: 'Show Fonts' allows you to change fonts and sizes using a standard font dialogue.
Font style: Bold, Italic and Underline can be directly specified.
Bigger/Smaller: steps font size up or down.
Copy/Paste Style: the style (without changing text) can be copied or pasted.
Rotation: see Rotating Text Objects for details of how to rotate the text.

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Resizing Text Objects
The width of text objects can be changed by dragging the handles that determine the size of each text object. These can be made to appear by clicking on the text object. When the width of a text object has been changed, the height of the object is automatically adjusted to abut the bottom of the text. If the text that is being resized is rotated, the text temporarily drops down to a vertical position so that you can see how this will affect text layout. Carry on resizing by dragging the cursor to where you want the edges of the text to be, and the dimensions will be reproduced on the unrotated text; once you release the mouse, the text will flip back to its rotated position.

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Rotating Text Objects
When one or more text objects are selected (by clicking on them with the mouse so the bounding rectangle and the resize handles appear), the text can be rotated using the *Rotate Text XX° Clockwise* or *Rotate Text XX° Anti-Clockwise* commands that are found in the *Text* menu. The default rotation step size is 45°, but this can be changed simply by entering a new value after using the *Change Rotation Step Size…* command in the *Text* menu. The new Rotation Step Size will be stored when you save the document. To completely unrotated the selected text object, use the *Revert To Unrotated Text* command in the *Text* menu.

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Miscellaneous

Keeping Notes
GelEval allows you to keep a notepad associated with each GelEval image file. This can hold any information you wish, such as a description of the gel and comments on how quantification was performed. The contents of the notepad are read by the Spotlight search system, so Spotlight can then be used to find relevant GelEval files. To open the notepad, use the *Show Notepad* command from the *Window* menu. Enter your notes onto the pad as you would for any standard word processor. The notepad is automatically saved with the GelEval custom file (see '**Saving the Quantification Data as a GelEval File**'). When an image file is first converted to a GelEval file, the location of the image file, its file type and its creation date are added to the notes.

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Printing
The most important GelEval windows can be printed: the main image plus the quantitation rectangles, the Quantification Table and the graph windows. Use the *Print...* command in the *File* menu or press ⌘*P*. The Page Setup properties can be accessed using the *Page Setup...* command in the *File* menu or by press <*shift*>⌘*P*. If the Page Setup is changed when a graph window is highlighted (using *File* > *Page Setup...* or <*shift*>⌘*P*), the Page Setup is applied to all the graphs associated with that gel. All GelEval quantification windows can also be saved to file using the *Export Window…* command from the *File* menu. This saves the uppermost window in appropriate file format: pdf for all windows except the Quantification Table whose data is saved as tab-delimited text (.txt).

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Undo/Redo
GelEval supports standard Macintosh Undo/Redo for all actions that change the way that the data is analysed. This includes rectangle creation, deletion and manipulation, and changes to the way that densities are calculated. In contrast, once graphs that have been plotted, their data is fixed, even if the rectangles used to define the graphed area have been changed. Undo/Redo is performed by selecting *Undo* or *Redo* from the *Edit* menu or by pressing ⌘*Z* (for Undo) or <*shift*>⌘*Z* (for Redo).

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Graphs
GelEval displays graphs in several contexts (vertical scans, the line fit data, and in the rectangle specifications screen). These graphs have some interactive capability:

If you double-click either the X axis or the Y axis, a sheet will appear, offering you the option to change the start and end positions of the axis, and to change the number of major and minor ticks. Any of these values can be edited; if you want them to be automatically determine, click the ‘Use Defaults’ button.

If you double-click anywhere else on the graph (ie away from either of the axes), a sheet will appear allowing you various options. ‘Show line names’ sets whether the data type plus associated graph symbols are displayed at the top of the graph. ‘Show live mouse coordinates’ sets whether the X,Y coordinates are displayed under the mouse, as described in **Live Graph Coordinates**. ‘Copy graph to the clipboard’ copies the data in the graph onto the clipboard, which can then be pasted as text into spreadsheet (eg Excel or Numbers) or word processing (eg Word or TextEdit) documents as test, or as a pdf into drawing programmes (eg Preview or Illustrator); for more details, see **Exporting Graphs**. ‘Show graph statistics’ brings up a new window displaying mean, mode and median values for the intensity, with an option to copy the data onto the clipboard.

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