



ScanAlyze User Manual

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NEW KEY COMMANDS (version 2.35+)

Navigating and Flagging Grids:

In the Image window, when there are Grids, the < and > keys allow you to navigate between grids.

If you press < or > [without shift or Ctrl] you will switch between Grids, and the screen will be centered around the center of the current grid. This grid (and only this grid) will be selected to allow you to adjust or refine the position of this grid.

If you press < or > with the Ctrl key depressed, you will similarly switch between Grids, but the grid will not be selected, and you will be in Select By Spot mode. This will allow you to flag spots on this grid.

Flagging can now be done, by selecting spots and pressing the 'f' key to flag and the 'u' key to unflag selected spots (this is best done combined with Ctrl-< and Ctrl-> navigation of grids.

Other Commands:

Dragging out a square with the Ctrl and left mouse button will zoom in on the marquee'd region.

Pressing Ctrl-a will adjust window size and position to optimally fit on the screen.

Current Version 2.35 (October 30,1999)

Version Information:

2.31 Added autoflagging.

2.32 Corrected bug in AutoFlagging.

Added correction to AutoFlag form to deal with European computers that use “,” instead of “.” In floating point values.

2.35 Added multiple new features for navigating between grids.

Description:

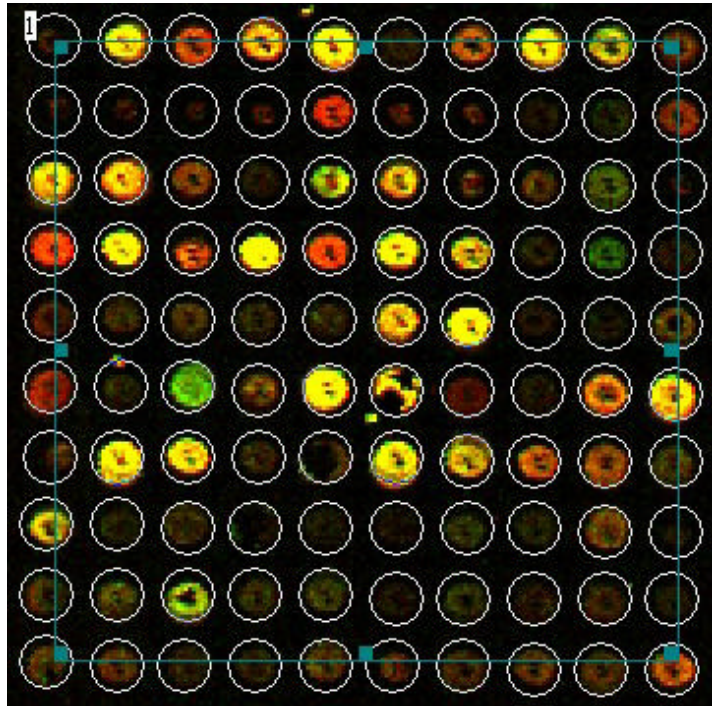
ScanAlyze is a program for the analysis of DNA microarray images. It operates on fluorescent images from single or two-color fluorescent hybridizations and produces a table of results for subsequent analyses.

Overview of operation:

Microarrays are constructed by robotically spotting DNA onto a solid substrate. Robots for producing microarrays can be built, using plans available at <http://www.microarrays.org/BuildYourOwnArrayer.html>, or purchased from a variety of commercial sources (see <http://www.microarray.org/BuyAnArrayer.html>).

To increase throughput, most arrayers use multiple printing tips. The image of a single tip (SECTOR) generally consists of rectangularly or hexagonally arrayed DNA elements (SPOTS). In order to extract data from microarray images, it is necessary to accurately identify the location of each of the spots.

ScanAlyze provides an interactive graphical environment for performing this process – GRIDDING – and provides tools to simplify and semi-automate this process. Following gridding, *ScanAlyze* extracts information on each spot (fluorescence intensities, background intensities, fluorescence ratios and a wide variety of quality control parameters) and saves a data file for use in subsequent analyses.



Tutorial:

When ScanAlyze is started, you will see a splash screen for 5 seconds (look for messages about available updates in the splash screen). You will then see the *Image Control* form. Use this window to load image files and to control how the image is displayed.

Loading Images

ScanAlyze supports two image formats:

- 1) A raw image file (.SCN) used at Stanford University
- 2) Standard 8- and 16-bit TIFFs (all images are stored internally as 16-bit)

Separate files are required for each fluor analyzed (ScanAlyze currently supports one or two images per array; future versions will support arbitrary numbers of images).

Images are loaded by clicking on the **Load** button corresponding to the appropriate channel. Corresponding to conventions used by most researchers, by default Channel 1 is considered to represent a green fluor (e.g. Cy3) and Channel 2 is considered to represent a red fluor (e.g. Cy5).

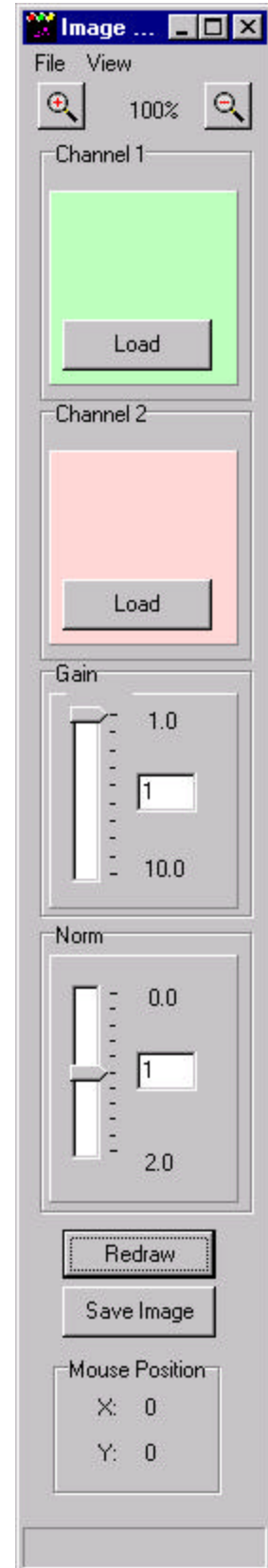
Displaying Images

After loading image files, click the **Redraw** button. A pseudocolor image of the array will be displayed using the default parameters.

On the Image Control form, the user can adjust the Gain and Normalization used to display the image. The default pseudocolor representation of the images represent every pixel by an RGB color value. With initial settings, the RGB values are

$$\begin{aligned}\text{Red} &= \text{Gain} * (\text{Ch2PixelInt} / 256) / \text{Normalization} \\ \text{Green} &= \text{Gain} * (\text{Ch1PixelInt} / 256) \\ \text{Blue} &= 0\end{aligned}$$

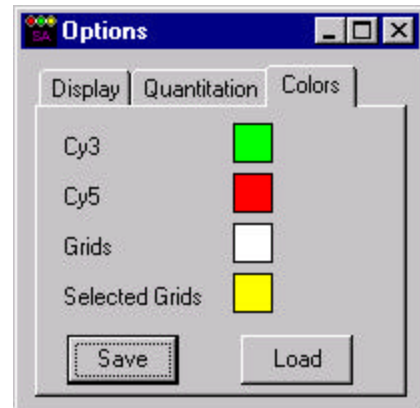
The *Gain* value controls the brightness of the image, and the *Normalization* value controls the balance between the two images (more below).



Display Options

Selecting the **View|Options** menu item brings up advanced display options.

The *Colors* panel allows the user to control which colors are used to represent each fluorescent channel in pseudocolor images (it also allows the user to display how grids are colored; this will be discussed below). By default, channel 1 is represented with pure green (RGB = 0,255,0) and channel 2 is represented with pure red (RGB = 255,0,0). Any alternate color scheme can be selected by the user (this feature was added for use by color-blind researchers).



Under the *Display* panel, you will see a pull-down menu with different image rendering methods.

Normal is the pseudo-color image used by default.

The displayed RGB value for each pixel is

$$Red = Gain \left(\left(\frac{Ch1PixInt}{65536} \right) Ch1Red + \left(\frac{Ch2PixInt}{Norm * 65536} \right) Ch2Red \right)$$

$$Green = Gain \left(\left(\frac{Ch1PixInt}{65536} \right) Ch1Green + \left(\frac{Ch2PixInt}{Norm * 65536} \right) Ch2Green \right)$$

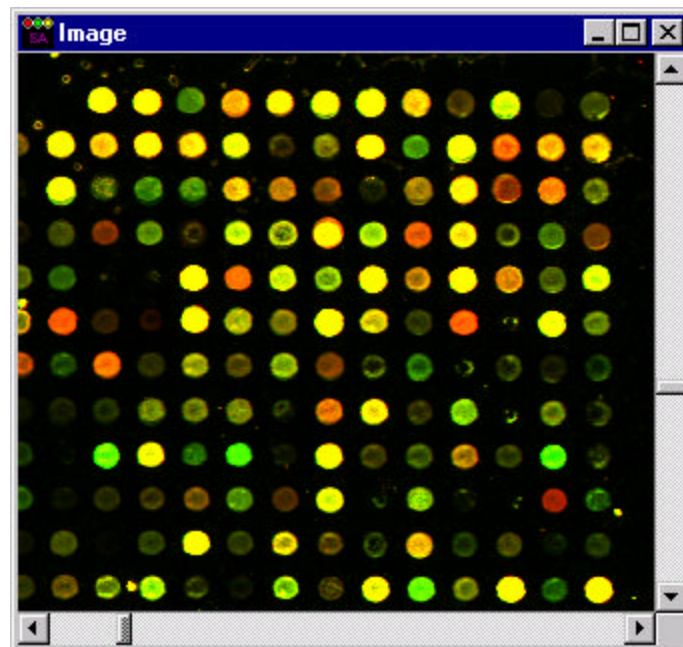
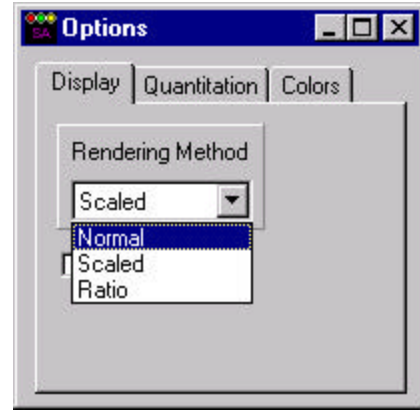
$$Blue = Gain \left(\left(\frac{Ch1PixInt}{65536} \right) Ch1Blue + \left(\frac{Ch2PixInt}{Norm * 65536} \right) Ch2Blue \right)$$

where

Ch1PixInt and Ch2PixInt = single channel pixel intensities (16-bit),

Ch1.Red, Ch1.Green, Ch1.Blue = user-selected RGB color for channel 1

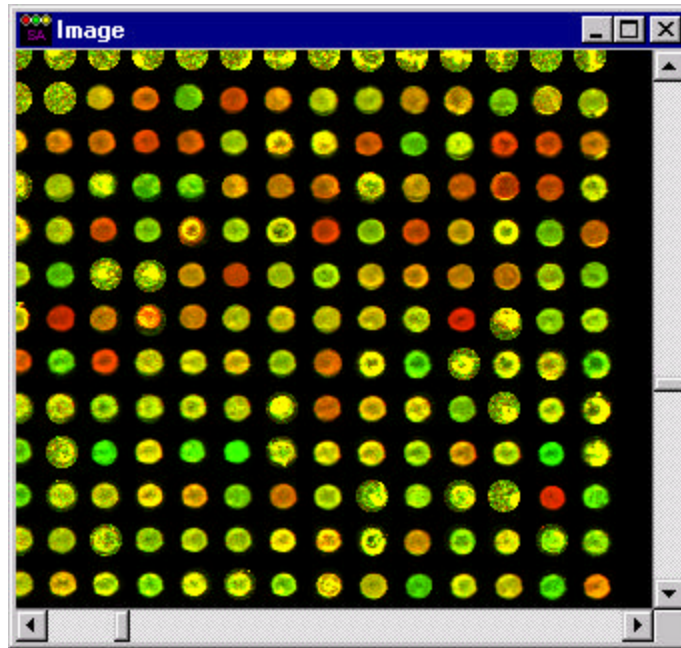
Ch2.Red, Ch2.Green, Ch2.Blue = user-selected RGB color for channel 2



Normally Rendered Image

After gridding and calculation of spot values, **Scaled** produces an image like that in **Normal**, except that a separate gain is computed for each spot ($\text{SpotGain} = \text{Gain} * \text{Median Pixel Intensity in spot}$). Background pixels are colored black.

This is intended to allow all spots to be viewed on a roughly equivalent scale, although it produces aesthetically unappealing results for weak spots.



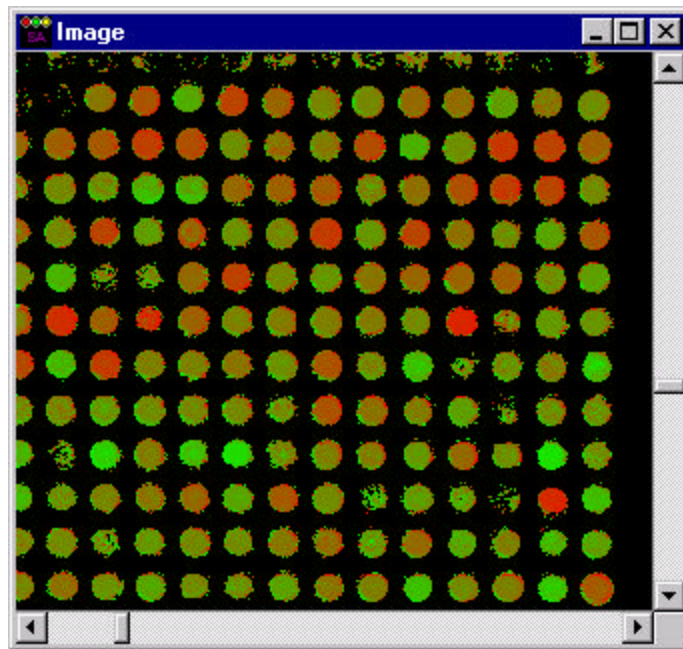
Scaled Image

Ratio produces an image where the Ch1 and Ch2 colors are mixed based on the single pixel Ch1/Ch2 ratio.

$$Re\ d = \left(\left(\frac{Ch1PixInt}{Ch1PixInt + \frac{Ch2PixInt}{Norm}} \right) Ch1\ Re\ d + \left(\frac{\frac{Ch2PixInt}{Norm}}{Ch1PixInt + \frac{Ch2PixInt}{Norm}} \right) Ch2\ Re\ d \right)$$

Green and Blue computed similarly with Ch1Green, Ch2Green and Ch1Blue, Ch2Blue
 Navigating Images

If background values have been calculated, Ch1PixInt and Ch2PixInt are background corrected and background pixels are excluded.



Ratio Image with
background correction

Navigating Images

As with all Windows applications, only the portion of the image that fits in the ImageForm is shown. Users can navigate through the image using the horizontal and vertical scroll bars. Users can zoom in or out using the + or - buttons on the ImageControlForm or by pressing Ctrl+ or Ctrl- in the Image window. The zoom can be reset to 100% by clicking on the magnification label (where it says ___%) on the ImageControlForm.

Gridding

The most important step in processing an array is gridding - where the location of spots and their position in the regular array of DNA elements are identified.

Although, in principle, this step could be done automatically, in practice it remains an interactive process (although we are working on it).

When you display an image, the *Grid Control Form* is automatically displayed.



Creating New Grids

The first time you analyze an array from a batch (in general, arrays are printed in multiples of 50 to 150 identical arrays) you need to create a new grid. To do this you click on the *New Grid* button on the *Grid Control Form*, bringing up the *New Grid Form* shown here.

Using this form, between 1 and 32 new grids can be created. First the number and orientation of spots in each grid/sector must be entered:

Columns: number of columns of spots in each grid

Rows: number of rows of spots in each grid

The size of each spot (currently in pixels) should also be entered in **Spot Width** and **Spot Height**.

The spacing between the center of these spots should be known from how the arrays were generated, and these values should also be entered (if not known, provide approximate values).

Col Spacing: horizontal spacing (um) between spots

Row Spacing: vertical spacing (um) between spots

To convert these values into pixel coordinates on the image, ScanAlyze needs to know the resolution of the image. Often, these are determined automatically from the image file. If incorrect, enter values:

Xres: horizontal size of each pixel (um)

Yres: vertical size of each pixel (um)

Where multiple printing tips were used, multiple identical grids can be added at once. **Tip Spacing** defines the offset distance (um) between. Currently, if 4 grids are added in a 2x2 pattern, 16 in a 4x4 pattern, and 32 in a 4x8 pattern. Future versions will support arbitrary layouts of grids.

For example, for the test images provided, 16 printing tips were used, in a 4 by 4 configurations, spaced at 4.5 mm apart. Each grid/sector contained 18 columns of spots and 16 rows of spots. The spot centers were spaced 223 um apart. The image was scanned at a resolution of 10 um. Based on manual inspection of the image, the spots have a diameter of approximately 14 pixels and the upper-left-hand spot on the array had center coordinates of X=85 and Y=88 (determined by moving the mouse over the spot).

The screenshot shows a dialog box titled "New Grid". It has a title bar with standard window controls (minimize, maximize, close). The dialog is divided into several sections:

- Grids To Add**: A group box containing four radio buttons labeled 1, 4, 16, and 32. The 16 radio button is selected.
- Columns**: A text input field containing the value 18.
- Rows**: A text input field containing the value 17.
- Col Spacing (um)**: A text input field containing the value 223.
- Row Spacing (um)**: A text input field containing the value 223.
- XRes (um)**: A text input field containing the value 10.
- YRes (um)**: A text input field containing the value 10.
- Tip Spacing (um)**: A text input field containing the value 4500.
- Spot Width**: A text input field containing the value 14.
- Spot Height**: A text input field containing the value 14.
- First Grid Position**: A section with two text input fields: "Left" containing 85 and "Top" containing 88.
- At the bottom, there are two buttons: "Create" and "Cancel".

The new grids will be drawn on the image. If the entered parameters were correct, the grids should look pretty much like the array. However, because the printing and scanning processes are inexact, some manipulations are necessary. All parameters of a grid can be adjusted, except for the number of columns and rows of spots (if this is entered incorrectly, you must delete the grids and start over). Six adjustable parameters define each grid, the coordinates of the center of the upper left spot (**Left,Top**), and two vectors that define the column and row spacing, (**Col.X,Col.Y**) and (**Row.X,Row.Y**). The center of each spot in the grid (in column position i and row position j), is set to

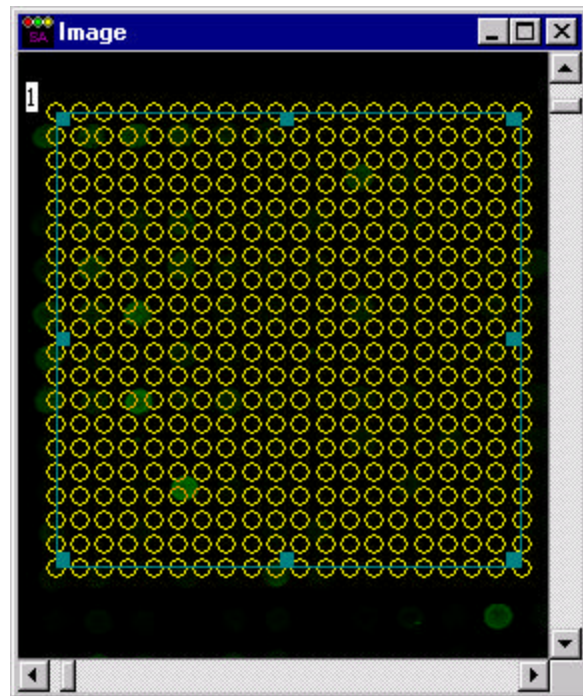
$$X_{i,j} = Left + i * Col.X + j * Row.X$$

$$Y_{i,j} = Top + i * Col.Y + j * Row.Y$$

[Individual spots can be offset from this position; this will be described below]. When new grids are created Col.Y and Row.X are 0, meaning that the grid axes are parallel to the image axes.

ScanAlyze provides three methods for adjusting these parameters.

Handles: Each grid is associated with a grid box, which in turn has eight handles. These can be used to move or stretch the grid. To move, simply left-click on the grid and drag, the grid box will move with the mouse. When the mouse button is released, the grid will be moved. This action adjusts only **Left** and **Top** based on to where the grid box was dragged. To stretch, position the mouse over one of the small square handles along the edge of the grid. When a directional arrow appears, left-click and drag to stretch or compress the grid. When Col.Y and Row.X are not zero, the results may be counterintuitive at first. When the mouse button is released, Left and Top are adjusted (if the left and/or top edge of the box is moved) and then the column and row vectors are altered based on changes in the width and height of the grid box. Col.X and Col.Y are scaled by the ratio of the old height to the old width, and Row.X and Row.Y are scaled by the ratio of the new height to the old height.



Directional Buttons: The parameters that define a grid can be manipulated with the directional buttons on the Grid Control Form. These buttons do different things depending on which action button is pressed. For these buttons to have an effect, grids or spots must be selected. Three selection modes exist. If you want to move individual grids, set the *SelectBy* radio button to *Grids*. Grids can then be selected/deselected by holding down the shift key and either left-clicking on the grid or dragging a select box over some portion of the grid. If you want to select all grids set the *SelectBy* radio button to *All*. Right click outside of a grid to deselect all, or click on the *Unselect* button. When operating in either of these modes, all five *ButtonActions* are available. Their actions are as follows. A left-click on a button generates a “small” action, a right-click a “large” action.

Move: Buttons move the entire grid. Up/Down adjusts Top. Right/Left adjusts Left.

Stretch UL: Buttons stretch the grid with bottom-right fixed. Up/Down adjusts Top. Right/Left adjusts Left. Column and Row vectors adjusted to keep Bottom/Right of grid box fixed.

Stretch BR: Buttons stretch the grid with upper-left fixed. Up/Down scales RowX and RowY. Left/Right scales ColX and ColY.

Tilt: When *SelectBy = Grid*, the Up/Down Buttons tilt each grid about their upper-left corner by rotating the Column and Row vectors. When *SelectBy = All*, the Up/Down Buttons tilt each grid about their upper-left corner by rotating the Column and Row vectors, and rotate the upper-left corner of each grid about the upper-left corner of grid number 1. In Tilt mode, the Left/Right buttons do nothing.

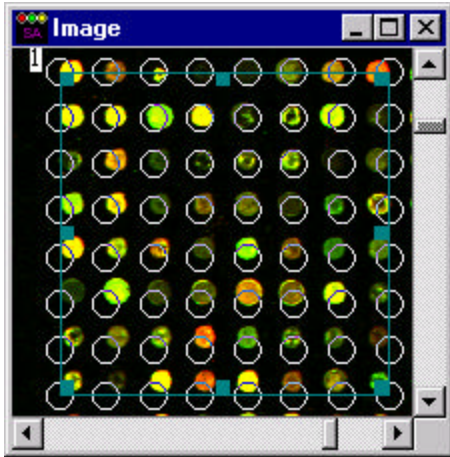
Warp: Adjusts only Column vector to allow angle between Column and Row vector to be non-orthogonal. Up/Down buttons adjust ColY. Left/Right buttons adjust ColX.

Hint: The arrow keys on your keypad act like the buttons on the form.

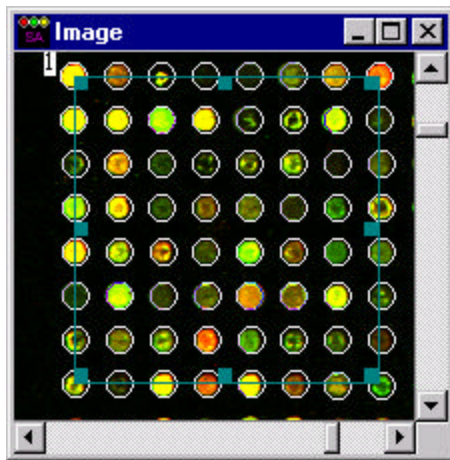


Refinement:

When the grid is close to its proper orientation, ScanAlyze can optimize the parameters automatically. Simply select the grid and click the **Refine** button. This option is under constant development, but the implementation in the released version has performed well on most arrays. Each time you hit Refine, the parameters are adjusted a small amount. Multiple cycles are usually necessary. An example is shown below.



Before Refinement



After Refinement

Adjusting Spot Position

In some cases, individual spots are displaced relative to their inferred position on the grid. This often occurs when printing tips move during the printing process. To allow such arrays to be properly analyzed, ScanAlyze allows individual spots to be offset relative to the grid. Internally, each spot carries with it ColOffset and RowOffset parameters. These are offsets along the Column and Row vectors, and are stored as percentage of the length of the vector. For spots where ColOffset and RowOffset are not zero, the actual spot center is located at

$$X_{i,j} = Left + (i + ColOffset) * Col.X + (j + RowOffset) * Row.X$$

$$Y_{i,j} = Top + (i + ColOffset) * Col.Y + (j + RowOffset) * Row.Y$$

Individual spot positions are adjusted by setting the SelectBy radio button to Spots. Spots are selected/deselected by holding down the shift key and either clicking on individual spots or dragging a selection rectangle over the spots. All selected spots can be unselected by right-clicking outside of a grid box or by clicking the Unselect button.

When in Spot selection mode, only the Move action is available. Up/Down buttons adjust RowOffset. Left/Right buttons adjust ColOffset.



Adjusting Spot Size

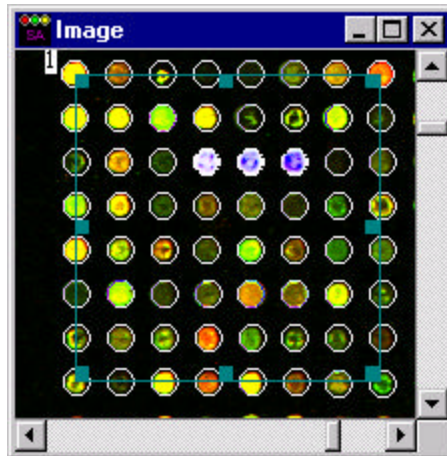
In ScanAlyze, spots are represented by ellipses. The size of each spot can be adjusted after grids are created. Simply select the spots you wish to resize, enter new values for spot Width and Height in the ResizeSpots panel of the GridControlWindow and hit the Set button. If you are in Grid select mode, all spots in selected grids will be resized. If you are in Spot select mode, only selected spots will be resized.



Note: ScanAlyze handles spot size internally with a grid parameters SpotWidth and SpotHeight, and spot parameters SpotWidthOverride and SpotHeightOverride. When grids are created, SpotWidth and SpotHeight are set to the specified values and SpotWidthOverride and SpotHeightOverride are set to 0. The size of a spot is equal to SpotWidth x SpotHeight, unless the override values are non-zero, in which case the spot is SpotWidthOverride x SpotHeightOverride. When spots are resized in Grid select mode, SpotWidth and SpotHeight are altered. In Spot select mode, SpotWidthOverride and SpotHeightOverride are altered. Thus, if the size of a single spot has been changed, and the spot size is changed in Grid select mode, the size of the previously changed spot will not be affected. To reset spot sizes to the grid specific values, select all spots in the grid in Spot select mode, and set the Width and the Height to 0.

Flagging Spots

Often, users will want to flag certain spots for specific handling in subsequent analyses, especially spots where the values will likely be corrupted by problems on the array. ScanAlyze allows to user to assign an integer flag to selected spots. Select spots in Spot select mode, specify the flag value and hit the **Flag** button. Spots start with a flag value of 0. All spots where Flag is not equal to zero are displayed as a solid ellipse.



Spots can be unflagged by selecting them and setting their Flag value to 0.

Automatic Flagging of Spots

A new option (implemented in versions 2.31 and beyond) allows the user to specify cutoff values for automatic flagging of spots. This option only works after the spot parameters have been computed (by pressing the *SaveData* button). Select the grids to which you wish to apply autoflagging, and choose autoflagging parameters by checking the appropriate box. Enter the desired cutoff value and press *Apply*. The spots that do not pass the cutoff values are highlighted with a bold outline. If you wish to make these flags permanent, hit *Accept*; otherwise change the parameters and hit *Apply* again to change the values.

Checkbox	Label	Value
<input type="checkbox"/>	Ch1D	100
<input type="checkbox"/>	Ch2D	100
<input type="checkbox"/>	Ch1B	100
<input type="checkbox"/>	Ch2B	100
<input type="checkbox"/>	Ch1D/B	0.5
<input type="checkbox"/>	Ch2D/B	0.5
<input checked="" type="checkbox"/>	Ch1GTB1	0.55
<input checked="" type="checkbox"/>	Ch2GTB1	0.55
<input type="checkbox"/>	Ch1KSD	0.5
<input type="checkbox"/>	Ch2KSD	0.5

Grids can be saved and loaded with all of their parameters intact using the Save Grids/Load Grids buttons. They are stored in a binary file format with the extension SAG (ScanAlyzeGrid). Specifications are available upon request.

When arrays are made in large batches, as they usually are, many features of the arrays are consistent across the batch. Therefore, after gridding the first array from a batch, it is generally possible to use this initial grid for all subsequent arrays in the batch, with only minimal readjustment or refinement.

The grids, handles and associated labels can be turned on or off using the appropriate Hide/Show toggle buttons. On slower machines, it slows down the program when a large number of grids are shown. The users may wish to perform some operations with the grids hidden.



Recommended Procedure for Gridding Arrays

- 1) Load Images
- 2) Adjust Gain so the most spots are visible against the background
- 3) Create all grids at once with values obtained from array manufacture
- 4) Hide grids
- 5) Move and stretch each grid box so that the corners are close to their appropriate location on the image
- 6) Show grids
- 7) Save grids
- 8) Refine (either by selecting one grid at a time or by selecting all grids) until positions look correct
- 9) Save grids
- 10) Move individual spots whose positions need correction
- 11) Save grids
- 12) Flag spots in problem areas
- 13) Save grids
- 14) Calculate and save data (see below)

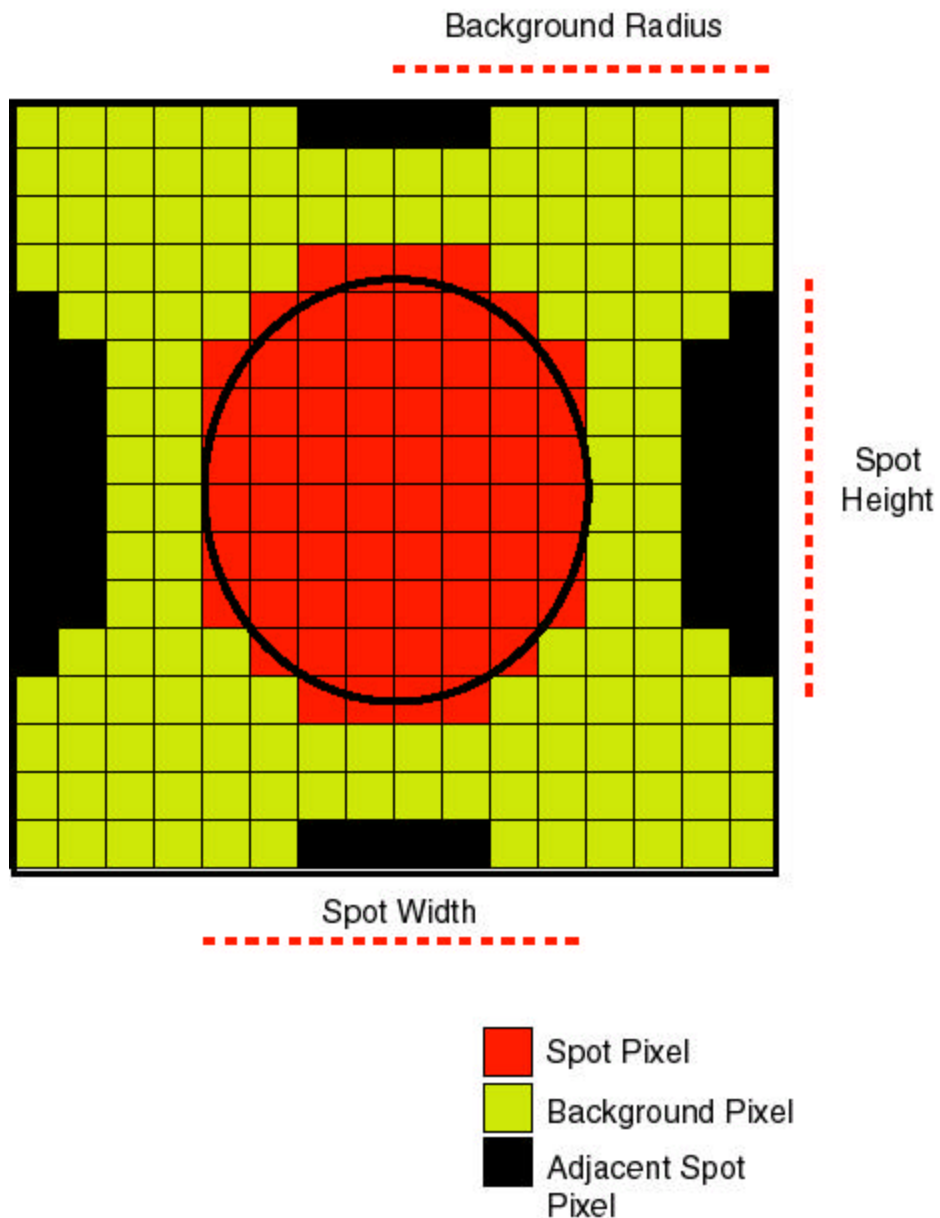
Steps 1-9 take an experienced user approximately 2 minutes on a reasonably fast computer. Flagging can be a time consuming process. Methods are being tested to automatically locate poor quality spots and will be included in future versions. Many quality control parameters are provided in the current version. By using these values, users may find most flagging unnecessary.

For gridding arrays from the same batch as a previously gridded array

- 1) Load Images
- 2) Adjust Gain so the most spots are visible against the background
- 3) Load Saved Grids from previous array in batch
- 4) Set SelectBy = All
- 5) Move and/or Tilt grids with Direction Buttons until they are roughly aligned with array image
- 6) Save grids
- 7) Refine all grids at once until positions look correct
- 8) Save grids
- 9) Move individual spots whose positions need correction if needed (if arrays in batch are consistent, this should be unnecessary)
- 10) Save grids
- 11) Check flags, and unflag/flag spots where necessary (many spots flagged on one array in a batch will also require flagging in all other arrays)
- 12) Save grids
- 13) Calculate and save data (see below)

Once the array has been gridded, hit the “Save Data” button. The program will then calculate information for each spot and save a tab-delimited text file with the results.

As the first step in the analysis, ScanAlyze separates the image into pixels that are contained within identified spot and those that are not. The diagram below illustrates this process for a single spot. Any pixel through which the spot circle passes is defined as being in the spot (red pixels). Any pixels not within the spot but which are within a square, centered at the spot center, with side lengths of $2 * \text{BackgroundRadius}$ pixels (a user-defined parameter that defaults to 20; in this diagram it is 8) are defined as background pixels for this spot (yellow pixels), excluding pixels contained within another spot (black pixels).



Before discussing the methods used to analyze microarray images, it is important to emphasize that the goal of this analysis is to accurately determine, for each spot, the *ratio* between channel 1 (green) and channel 2 (red) fluorescence. For a complete discussion of why ratios, and not fluorescence intensities, are the primary data used see M.B. Eisen and P.O. Brown, DNA arrays for analysis of gene expression, *Methods in Enzymology* 1999; 303:179-205.

The simplest form of analysis performed by ScanAlyze is to estimate the Ch1/Ch2 ratio based on the background corrected mean fluorescence intensities of the (red) pixels within the spot ellipse. First, the uncorrected mean pixel intensities are determined (exported in the datafile as columns **CH1I** and **CH2I**, along with a count of the number of pixels contained in the spot **SPIX**). Two methods for estimating the local background are provided. These are the median (**CH1B**, **CH2B**) and mean (**CH1BA**, **CH2BA**) intensities of the background (yellow) pixels (the number of background pixels used is exported in column **BGPIX**). We recommend the use of median background values for calculating ratios, as this measurement is less susceptible to noise. Some software also utilizes medians for determining spot intensities. We do not recommend this, and do not export this value; while we assume we are sampling from a uniform distribution for determining the background, this assumption is clearly invalid for spots where variations in amount of hybridizable DNA across the spot can lead to extremely non-uniform distributions. To compute ratios for use in subsequent analyses, simply compute

$$\frac{Ch1}{Ch2} = \frac{CH1I - CH1B}{CH2I - CH2B}$$

Although susceptible to errors due to stray fluorescence, on clean arrays the background this is as good an estimate as any for ratios provided that the net intensities are not too close to zero. A significant volume of data collected in our lab supports the reproducibility and biological significance of these values extracted from arrays examining a wide variety of processes in bacteria, yeast, mouse and humans. However, the mean pixel intensity can be susceptible to various types of noise and artifacts, and the accuracy of the measurement varies. ScanAlyze provides a number of different estimates of the ratio as well as quality control parameters that can be used to filter or weight spots.

While the amount of DNA often varies considerably across a spot, the background corrected pixel *ratio* should be fairly constant. ScanAlyze utilizes this property in alternate estimates of the ratio and spot quality. One alternate estimate of the ratio for a spot is the median of the set of background corrected single pixel ratios for all (red) pixels within the spot. Unlike pixel *intensities*, the background corrected pixel *ratios* are expected to be uniform, and thus the median is a useful value. The exported column **MRAT** contains the median of

$$\frac{Ch2PI - CH2B}{Ch1PI - CH1B}$$

where Ch1PI and Ch2PI represent single pixel intensities. The primary utility of this value is that it is less susceptible to artifacts that can corrupt mean intensity based ratios, such as bright fluorescent specks.

Two additional estimates of the ratio are based upon the assumption that, when single pixel intensities in channel 1 are plotted against intensities in channel 2, they will fall on a straight line with slope equal to the ratio. This assumption is based on a model for single pixel intensities that assumes a constant background in each channel and varying DNA content in each pixel. Thus, for a spot representing a gene with cognate probe in the hybridization solution having a red/green ratio of **R**, the channel 1 (green) and channel 2 (red) intensities at each pixel will be

$$Ch1BG+k * D \quad \text{and} \quad Ch2BG+ R *k * D$$

respectively, where **Ch1BG** and **Ch2BG** are the uniform backgrounds in the two channels, **k** is a constant and **D** is the amount of DNA in the region covered by the pixel. Thus, when channel 1 pixel intensities are plotted against channel 2 pixel intensities, they will fall along a line with slope k passing through the point (Ch1BG,Ch2BG). The exported columns **REGR** and **LFRAT** estimate the slope of this line by either simple linear regression of channel 2 on channel 1 (**REGR**) or a least-squares fit of a line (**LFRAT**) to the points, minimizing the sum of the squares shortest distance from each point to the line. The exported column **CORR** contains the correlation between channel 1 and channel 2 pixels within the spot, and is a useful quality control parameter (in general, high values imply better fit and good spot quality).

Additional quality control parameters are also included. These are

CH1GTB1 and CH2GTB1

Fraction of pixels in the spot greater than background (CH1B or CH2B)

CH1GTB2 and CH2GTB2

Fraction of pixels in the spot greater than 1.5 times the background (CH1B or CH2B)

On a perfectly clean image with no spotted DNA, roughly half of the pixels in a randomly placed spot should have intensities greater than the local background and few pixels much greater than this background. Thus, an empty spot will have CH1GTB1 and CH2GTB1 values of close to 0.50 and CH1GTB2 and CH2GTB2 values close to 0, while for uniformly bright spots these values should all be close to 1. This can be used to filter out weak spots and spots where the intensity comes from a few bright pixels (these will have high mean intensities but low values of these parameters). This value can be used to filter out weak spots or spots where the intensity comes only from a small number of bright pixels (e.g. dust). Cutoff values of between 0.55 and 0.65 are recommended.

An alternative method for capturing this information is output in the fields

CH1KSD, CH1KSP and CH2KSD, CH2KSP

Which compares the distribution of pixel intensities within the spot circle and in the background. The values CH1KSD and CH2KSD are the value of the Komogorov-Smirnov statistic that assesses the likelihood that the spot pixel intensity distribution is drawn from the background distribution. The actual probabilities are output in CH1KSP and CH2KSP. For more information on this test, see:

http://www.ulib.org/webRoot/Books/Numerical_Recipes/bookcpdf/c14-3.pdf

CH1EDGEA and CH2EDGEA

Mean magnitude of the horizontal and vertical Sobel edge vectors contained within each spot. These values are used during refinement. It is expected that a good spot should have relatively high mean edge scores, but we have not yet carried out a systematic analysis of these parameters behaviour.

Other exported columns

SPOT

Unique index of spot in file - counting starts with grid1, moves along row1 from column1 until the last column, then advances to the next row; after all rows in grid1 are assigned an index, counting proceeds to grid2, etc...

GRID,ROW,COL

Contain the grid in which the spot is contained, and the location of the spot within the grid.

LEFT,RIGHT, TOP,BOTTOM

Coordinates of box containing spot ellipse, in image coordinates.

FLAG

User defined spot flag (default 0)

NEW KEY COMMANDS (version 2.35+)

Navigating and Flagging Grids:

In the Image window, when there are Grids, the < and > keys allow you to navigate between grids.

If you press < or > [without shift or Ctrl] you will switch between Grids, and the screen will be centered around the center of the current grid. This grid (and only this grid) will be selected to allow you to adjust or refine the position of this grid.

If you press < or > with the Ctrl key depressed, you will similarly switch between Grids, but the grid will not be selected, and you will be in Select By Spot mode. This will allow you to flag spots on this grid.

Flagging can now be done, by selecting spots and pressing the 'f' key to flag and the 'u' key to unflag selected spots (this is best done combined with Ctrl-< and Ctrl-> navigation of grids.

Other Commands:

Dragging out a square with the Ctrl and left mouse button will zoom in on the marquee'd region.

Pressing Ctrl-a will adjust window size and position to optimally fit on the screen.