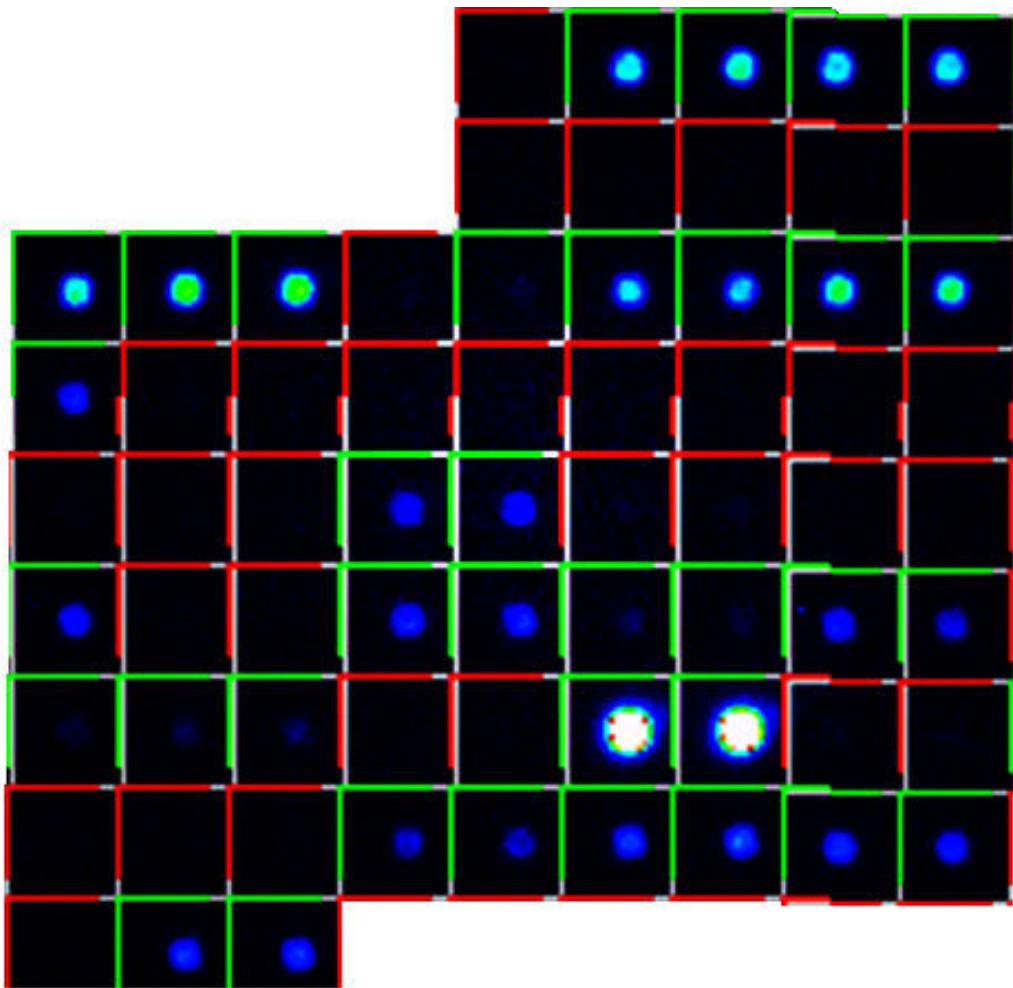




Genorama[®] Genotyping Software[™] 4.5



User Guide

For MS Windows XP

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Genorama® Genotyping Software™ 4.5 User Guide for Windows

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1 Introduction

Welcome to use the Genorama® Genotyping Software™ 4.5, a microarray image analysis software specifically designed for genotyping by Arrayed Primer Extension (APEX). This is a high-throughput genotyping method combining both high information content of oligonucleotide microarray and reliability of a genotyping method, based on single base primer extension. Our software is compatible with microarray images from primer extension assays combined with single, dual or four-colour detection.

The analysis using images from an APEX assay is performed as follows: four image files with signals from different dye terminators are opened. A grid is set up over the microarray**. The signals on the images are identified and quantified. The signal intensities on the same location (spot) are compared between different images and the base calls are made.

Genorama® Genotyping Software™ 4.5 contains two main utilities: BaseCaller™ for analysis of one sample at a time and PicDB™ for simultaneous analysis of multiple images by the same address.

* For more information on APEX method, please visit our homepage at <http://www.genorama.com/>

** Whenever multiple grids are located on the same image, they must be set up and analyzed separately

References:

Kurg A, et al. Arrayed Primer Extension: Solid phase four-color DNA resequencing and mutation detection technology. *Genetic Testing* 2000; 4(1):1-7

Tönisson N, et al. Evaluating the arrayed primer extension resequencing assay of TP53 tumor suppressor gene. *Proc Natl Acad Sci USA* 2002; 99(8):5503-8

BaseCaller™	PicDB™	Genorama® Converter™
Main program for analysis by single sample and to save image to database for analysis by PicDB™.	Main program by parallel analysis of multiple samples.	Converts tab delimited txt tables and 20-space blocks into Genorama reference tables (array positions with expected basecalls).



BaseCaller



PicDB



Genorama
Converter

1.1 About This Manual

The Genorama® Genotyping Software™ 4.5 User Guide provides detailed information about the tools and commands available. This manual is designed to be used as a reference tool in your everyday work with the Genorama® Genotyping Software™ 4.5.

This manual assumes you have a working knowledge of your operating system and its conventions, including how to use a mouse and standard menus and commands, and how to open, save, and close files. For

help with any of those techniques, please see your Microsoft Windows® documentation.

The manual is in PDF format. You can display the manual on your computer screen and print it out, if required. To access the manual you need to install the Adobe® Acrobat® Reader™ (<http://www.adobe.com/>).

1.2 System Requirements

To use the Genorama® Genotyping Software™ 4.5 you need the following hardware and software:

- An Intel® Pentium® – class or faster processor (Pentium IV with 1GHz CPU or better recommended)
- Microsoft Windows XP or later only.
- At least 128 megabytes (MB) of random-access memory (RAM) (512 MB recommended).
- A video card displaying minimum of 1024 x 768 pixels of desktop area.

Genorama® Genotyping Software™ 4.5 performance improves with faster versions of PC, more RAM, fast and large hard disk drives.

1.3 New in This Version

The new features in Genorama® Genotyping Software™ 4.5 are:

Genorama® BaseCaller™ 4.5 module

- New automatic grid placement module for image segmentation. After four clicks in the grid corner marks the software automatically locates signals and places grids on the four images.
- Genorama results window includes also PicDB section
- The field of notes and reference has unlimited characters space.

PicDB™ 4.5 module

- 2D clustering for automated genotyping is fully functional and linked with basecalls
- Both programs contain bug fixes for the older versions.
- The field of notes and reference has unlimited characters space.

Note: Microsoft Windows® XP is the recommended OS with Genorama® Genotyping Software™ 4.5

1.4 For Additional Information

Additional information about the software can be found in the following:

1. Web Site – <http://www.genorama.com/>
2. Sales Support by e-mail - info@genorama.com
3. Technical Support by e-mail - techsupport@genorama.com

1.5 Image Analysis Process

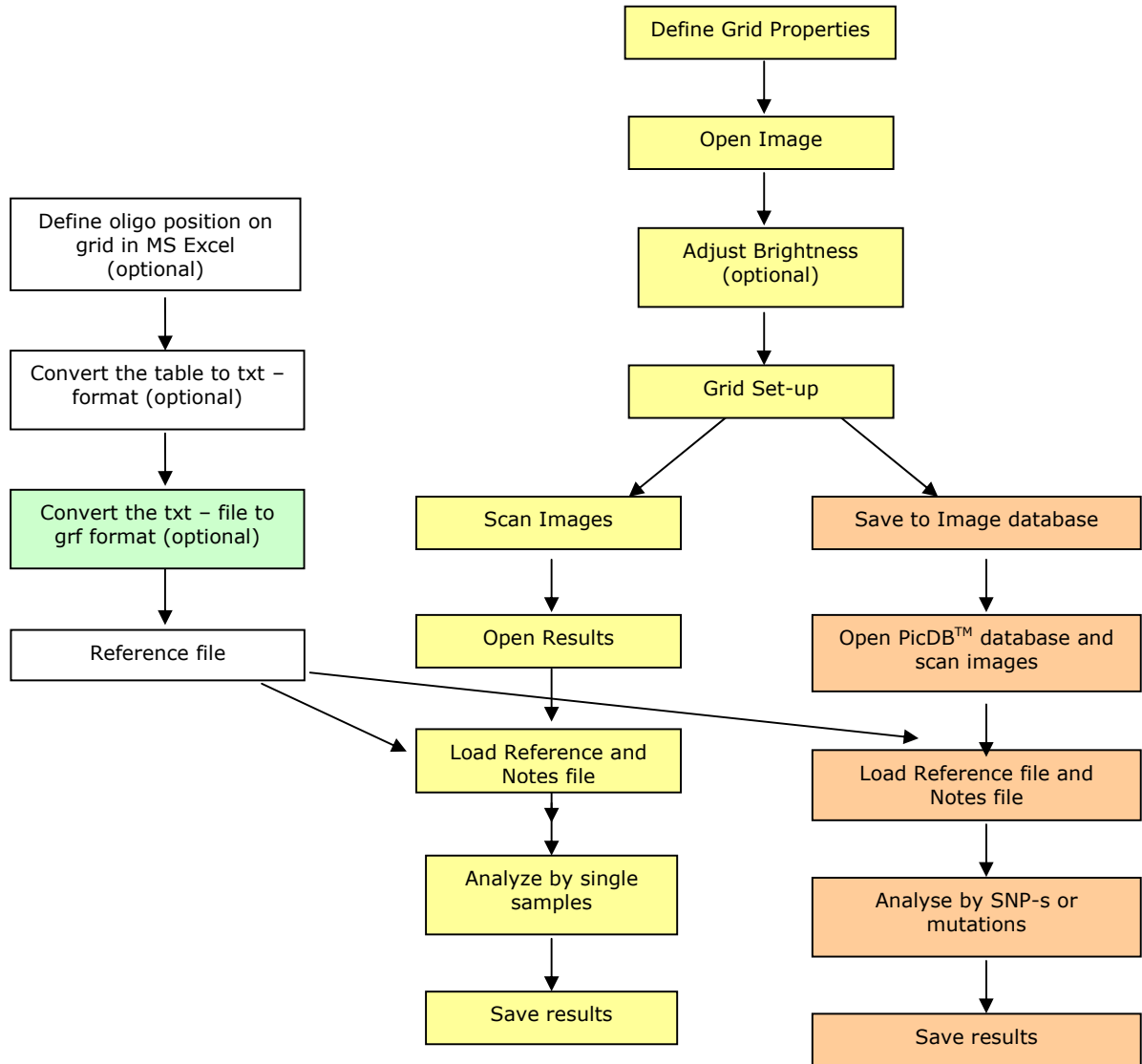


Figure 1. Image Analysis Process

2 BaseCaller™

2.1 Main Window



Figure 2. Genorama® BaseCaller™ Main Window

2.2 Working with Images

BaseCaller™ works with images saved in TIFF file format (Tagged-Image File Format). 12 or 16 bit digital APEX microarray images can be acquired with Genorama®

QuattroImager or with other microarray imaging systems.

2.2.1 Opening Images



Choose **Open image files** from **File** menu or click on the **Open button** on the toolbar. After adjusting Grid properties (See also section 2.2.1) the Open image files dialog box (See Figure 3.) appears.

BaseCaller™ lets you open up to four image files. To select and change image files click the **Browse** button.

Note: If you select a file with A, C, G or T in the end of the file name (e.g heart,056A.tif), BaseCaller™ automatically searches for and selects the other three images.

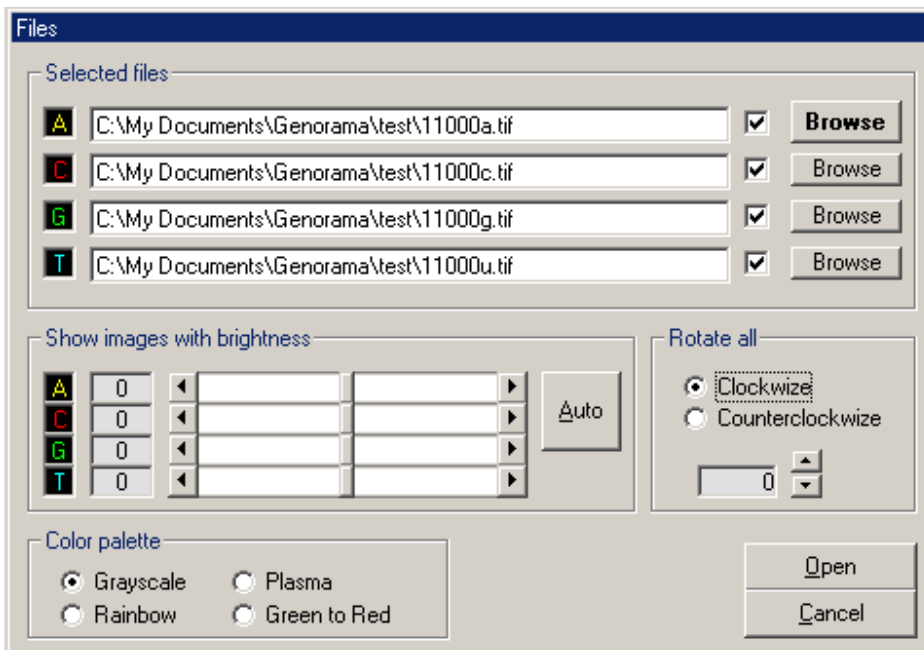


Figure 3. Open image files dialog box

With the Open command you can also adjust image brightness, rotate images and select image palette. Default settings are memorized from the last images opened.

2.2.2 Adjusting Image Brightness

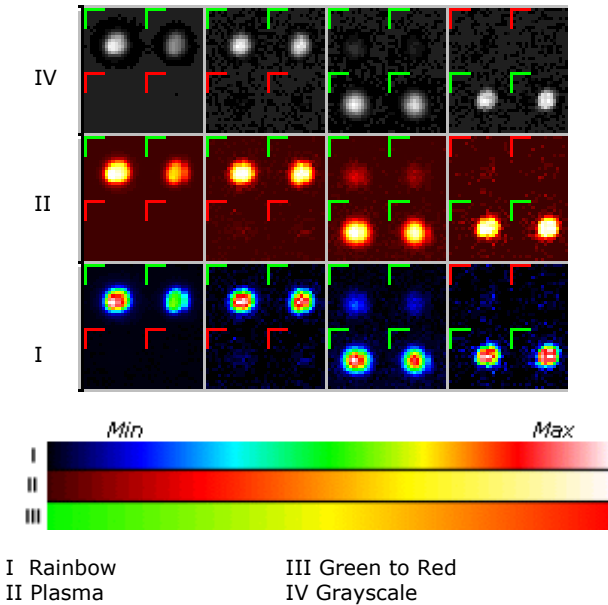


Figure 4. Different colour palettes

Using the Brightness command is the simplest way to adjust appearance of your image. You can change images' brightness and select palette.



Choose the **Brightness** command or click the **Brightness** icon on the toolbar.

Note: None of the changes have a permanent effect on your image files.

To adjust images' brightness do one of the following:

- Drag the sliders to adjust the brightness.
- Click the **Auto** button to set the overall brightness of images to the optimal level.

To rotate images do the following:

- Select 90 degrees and CW to rotate clockwise by a quarter-turn.
- Select 90 degrees and CCW to rotate counter-clockwise by a quarter-turn.
- Select 180 to rotate by a half-turn.

To select image palette do the following:

- Click the **Grayscale**, **Plasma**, **Rainbow** or **Green to Red** radio button to select image palette. Different image palettes (See Figure 4.) are for better visual identification and do not affect the results.

2.2.3 Magnifying and Reducing The View

You can magnify or reduce your view using various methods.



To zoom in do one of the following:

- Click the **Zoom in** button on the toolbar.
- Select exact percentage from the **Zoom** menu.



To zoom out do one of the following:

- Click the **Zoom out** button on the toolbar.
- Select exact percentage from the **Zoom** menu.

2.2.4 Using The Loupe Tool

The loupe tool lets you see the parts of the image in detailed view without magnifying the whole image.



To use the Loupe tool do the following:

- Choose **Loupe** from **Tools** menu or click the **Loupe** icon on the toolbar. The loupe window (See Figure 5.) appears.
- Move mouse around the image to see the detailed view inside the loupe window

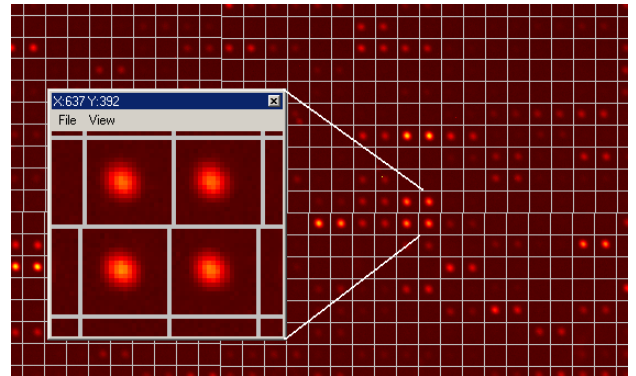


Figure 5. Loupe window

2.2.5 Using The Panorama Tool

The panorama tool shows your location on image and lets you move fast from one location to another



To Use the Panorama tool do the following

- Choose **Panorama** from Tools menu or click the **Panorama** icon on the toolbar. The panorama window (See Figure 6.) shows your exact location on image
- To move from one location to another click on the exact location on image.

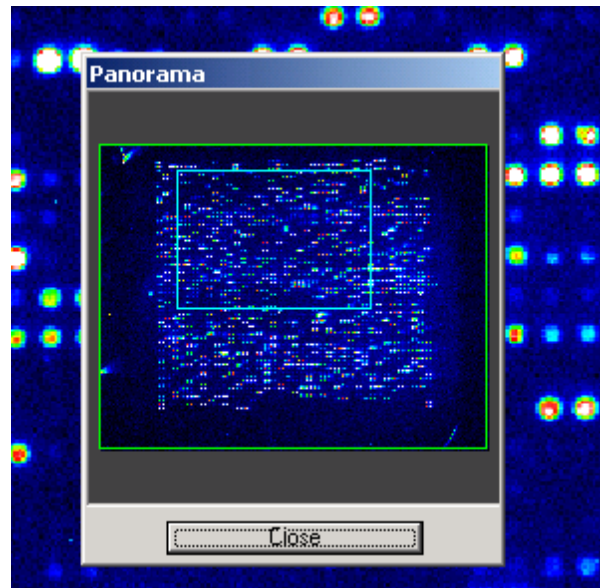


Figure 6. Panorama window

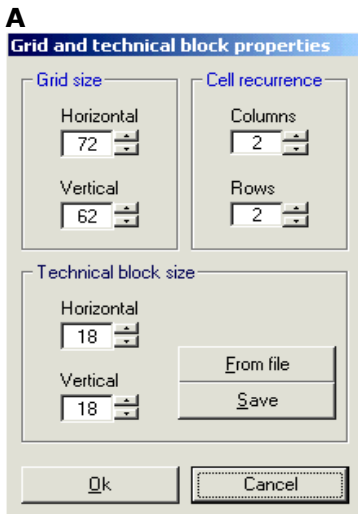


Figure 7A. Grid properties window

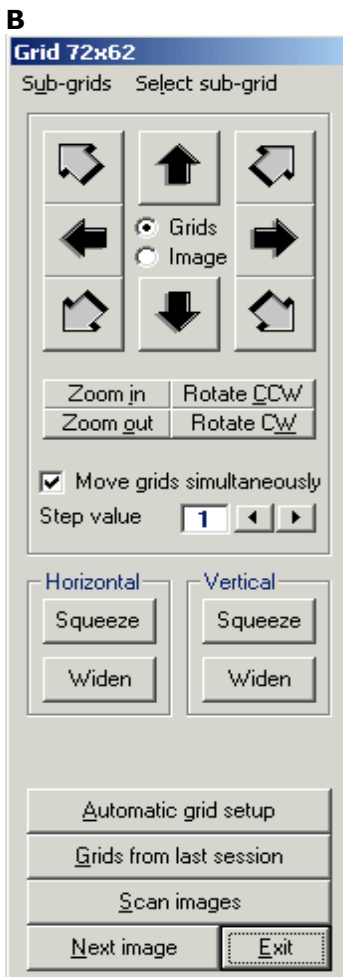


Figure 7B. Grid setup window

2.3 Setting Up Grids

2.3.1 Setting Grid Properties

In BaseCaller™ you set grid dimensions and grid cell recurrence immediately after the images are opened (See Figure 7A). Grid dimensions give the number of columns and rows of the grid. Grid Cell recurrence sets the number of identical neighbouring spots for the same oligonucleotide in the array. Technical block size sets the number of spots on array printed with a single pin. This allows correcting minor array deformations due to differences between the pins used for microarray printing.

Grid dimensions must fit the pattern of spots on the image.

To set up grid properties do one of the following:

Adjust the Grid size, Grid **Cell recurrence** and Technical block values to fit your image, click **OK** and proceed with grid setup (See also section 2.3.2).

To change grid properties do the following:

- Choose **Grid properties** from **Tools** menu. The Grid properties dialog box appears.
- Adjust the Grid size, Grid **Cell recurrence** and Technical block values to fit your image and click **OK**.

2.3.2 Setting Up Grids Automatically

To analyze the images a grid must be set up over the array area. The Grid tool lets you to segmentate the signals from regular array.



Choose **Grid setup** from **Tools** menu or click the **Grid setup** icon on the toolbar. Grid setup window appears (See Figure 7.).

Grid dimensions must fit the pattern of spots on the image. If you have set incorrect grid dimensions during the load time you have to correct them before setting up grids.

Click the **Automatic grid setup** button in the grid setup window. Select all four corners of the array. BaseCaller™ automatically checks the presence of signals on the selected positions and automatically places grid over the image.

After selecting four corners on array the program prompts you to confirm the technical block size. Select **Automatic setup** if you don't know the technical block size values. The program will automatically select values to compensate the array irregularities. **Select Manual** setup if you wish to set up the grids manually.

Note: Use the arrow buttons to scroll the view if necessary.

2.3.3 Setting Up Grids from last session

Grids from last session uses the grid size and location parameters from the last time the image was analyzed.

Click the **Grids from last session** button in the setup window. Grid appears on the image. Check grid size and location in each channel. Click the **Next image** button after each completed image.

Note: It is very important to set up grids correctly. Incorrect grid set-up affect signal quantification and can produce incorrect results.

2.3.4 Adjusting Grid Size and Location

BaseCaller™ lets you to adjust grid size and location using several tools. Use them if signals are not located in the centre of the grid cells.

Arrow buttons Use the arrow buttons to adjust the grid location. If the **Image** radio button is selected, you can scroll the view using the same arrow buttons.

Zoom buttons Use the zoom buttons to magnify or reduce the view.
Rotate buttons Use the **Rotate CW** button to rotate grid clockwise.

Use the **Rotate CCW** button to rotate grid anticlockwise.

Squeeze/Widen buttons Use the **Squeeze** button to reduce grid size. Use the **Widen** button to increase grid size.

Step value indicates the distance of grid movement in pixels each time an arrow button is clicked.

2.3.5 Setting Sub-Grids Manually

The sub-grid tool lets you manually divide the grid into sections. Use this tool to adjust each section separately, if your images have irregular spotting pattern, which is not corrected, by automatic grid placement.

To set up sub-grids do the following:

- Click the **Sub-grid** menu from the Grid setup window. The Sub-grid selection window appears (See Figure 8.).
- If you want to set up or change sub-grids, click the **Divide** button.

Vertical/Horizontal lines - Select **Vertical** radio button to draw vertical lines or **Horizontal** radio button to draw horizontal lines.

Up/down Left/Right buttons - Use those buttons to move line up/down or left/right by one step.

Cancel button - Click the Cancel button to discard your changes.

Reset button - Click the Reset button to remove all sub-grids.

OK button - Click the OK button to confirm your selection. To specify a part of grid to be adjusted, click on screen and the selected area become highlighted.

When you exit the part selection window the selected part remains highlighted in the main grid setup window. Now grid adjustments will be applied to the highlighted part of the grid.

To select a subgrid on the BaseCaller™ main window:

- Click **Select sub-grid** on Grid setup window (see Figure 7B). The Grid setup window will be temporarily closed.
- Click on the subgrid to be selected on BaseCaller™ main window.

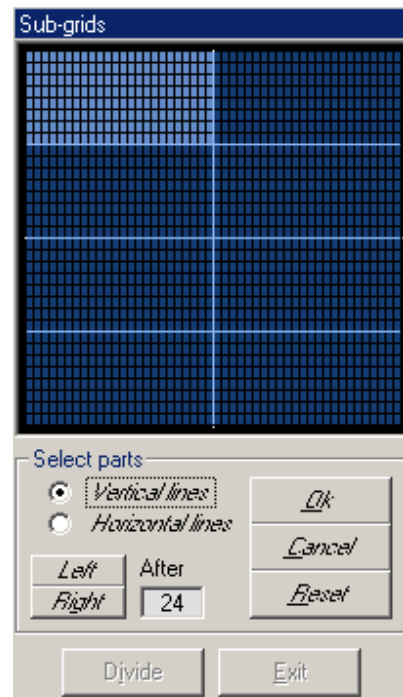


Figure 8. Sub-grid selection window

2.4 Creating and Opening Image Database

Image database is useful for retaining and comparing images, especially for single nucleotide polymorphism genotyping. Image database lets you open one position from each image and compare them across the database using Genorama® Genotyping Software™ 4.2.9 PicDB™ program (See also section 3.1).

To create a new image database do the following:

- Click the **Save to PicDB™** menu.
- Change image database folder, if necessary.
- Type the sample name in the textbox and
- Click **OK**.

To save images to the database do the following:

- Click the **Save to PicDB™** menu.
- Select your image database folder.
- Type the sample name in the textbox and
- Click **OK**.

NOTE: A new image database name is composed from grid dimension values. For example if your grid has 20 horizontal and 6 vertical cells the image database name is 20x6_1p.mdb. Additional databases-20x6_2p.mdb, 20x6_3p.mdb will be automatically created if a large number of images will be stored for the same grid size.

2.5 Scanning Images



First you must adjust image scanning and signal detection options. Choose **Options** from **Tools** Menu or

click the **Options** button on the toolbar. The options window appears (See Figure 9.)

2.5.1 Specifying Image Scanning Options

Choose between 2 signal detection algorithms:

- Cluster Analysis detects signal as the bright group of pixels (the only recommended option for 16 bit images)
- Gaussian Approximation finds signals according to geometrical distribution of intense pixels.

Valid diameter signals only - Use this option to eliminate small signals, probably artifacts.

Geometry Control - Use this option to eliminate signals that differ significantly from perfect circle, probably artifacts

Local background subtraction - Use this option to subtract the value of the background around the spot from the integrated volume of pixel intensities used for calculation of the signal intensity value.

Sensitivity - Use a sensitivity threshold to eliminate low intensity signals
 0% - only signals with high intensity are counted
 100% - all found signals are counted and quantified

Floating cells - Use this option for better recognition of the signals, which are not in the middle of the cell but are located on the border of the grid cell. BaseCaller™ searches area around the cell and automatically aligns the area for quantification according to the signal location. The area for this search is defined by the extent of border %.

0% - the actual cells within their visible border will be scanned.

30% - the actual cell plus area around the cell, 30% larger than the original cell, will be scanned. This improves signal recognition on arrays with moderately distorted columns and lines.

Show Floating Cells - Use this option to visualize the actual scanning alignment of cells.

Note: there are also options for blue cells, which are useful in results window (See also section 2.6)

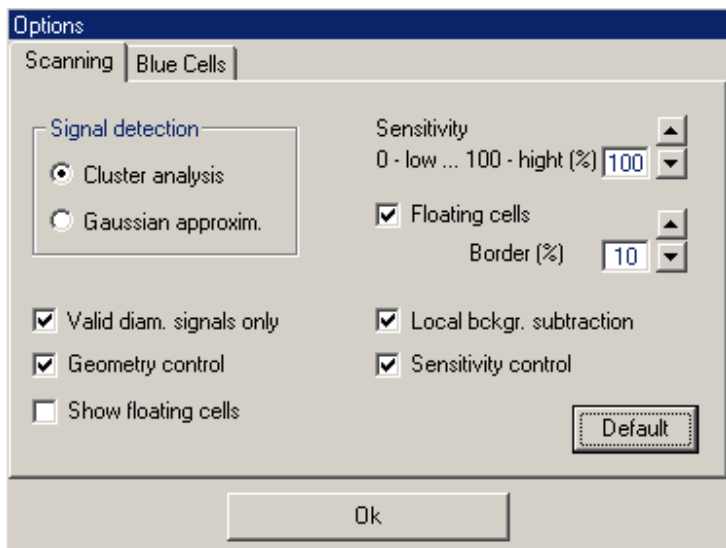


Figure 9. The Options window.



When scanning options are specified, choose **Scanning** from **Tools** menu or click the **Scanning** button on the toolbar or click the **Scan images** button in the Grid setup window. The Image Scanning window (See Figure 10.) appears.

2.5.2 Scanning Multiple Images

Click the **Scan all images** button to scan all images with the same scanning and signal detecting options.

Click the **Scan current image** to scan only one image at a time. This tool lets you specify different image scanning and signal detecting options for each image.

2.5.3 Eliminating Quantification of Weak Signals

When images are scanned and all signals are found, there may still be weak signals that may affect basecalls. **Intensity threshold** tool is used for eliminating these weak signals.

Use **Intensity threshold** tool as follows:

- Scan at least one image first.
- Click **Set**. The **Intensity threshold** window (See Figure 11.) appears.
- Choose a set of weak nonspecific signals by clicking on image to assign the threshold according to their **maximum** or **mean** intensity.
- Select, which images from **A, C, G** and **T** will be affected by the threshold set and the signals scan images again.

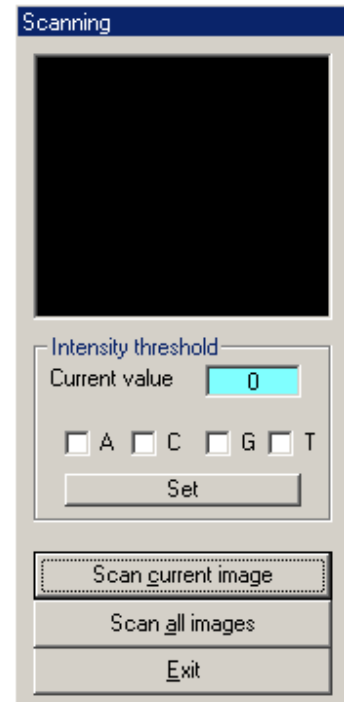


Figure 10. The Image Scanning window.

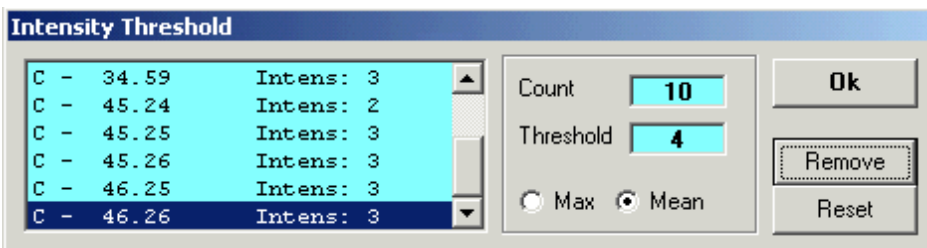


Figure 11. The **Intensity Threshold** window.

2.5.4 Visualising Signal Pixels

To visualize signal pixels of and view various pixel statistics press **Ctrl** and click on the scanned signal.

The signal- and background pixels with their parameters window (See Figure 12.) appears.

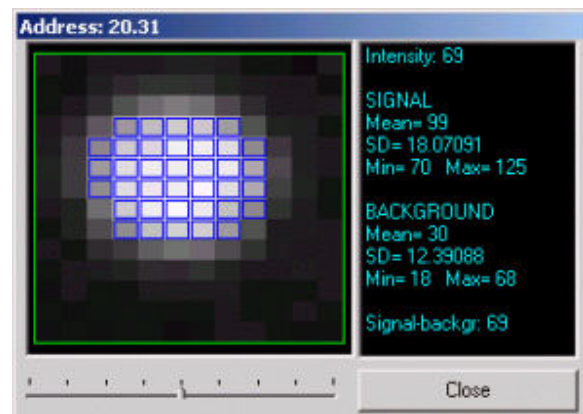


Figure 12. The signal- and background pixels with their parameters window

2.6 Sequence Analysis And Genotyping In Results Window



To open the **Results** window choose the **Results** menu on menu bar or click the **Results** button on toolbar. The Results window (See Figure 13.) appears.

The Results window gives you tools for performing APEX-based genotyping. BaseCaller™ supplies you with signal intensity values for each channel and preliminary basecalls in a 2D spreadsheet according to the intensity of each primer position in APEX microarray. You can add

notes, view detailed signal and reference information for a particular base or view a base pair if oligonucleotides for both DNA strands were arrayed. (For more information about APEX-based genotyping please read: "APEX – Arrayed Primer Extension", <http://www.genorama.com>).

Selected cell

The screenshot shows the 'Results' window with a table of base calls. A cell in row 5, column 5 (containing 'G') is highlighted and labeled 'Selected cell'. Below the table is a 'Reference and Notes window' showing 'U045se A G' and 'U045as T C'. To the right of this window are 'Load Refer' and 'Load Notes' buttons. Below the reference window are four image fragments labeled 'A', 'C', 'G', and 'T', with an arrow pointing to them labeled 'The image fragment of selected cell in each channel'. At the bottom right, a 'PicDB' section shows a list of samples with '0505320,H0103,260106' selected, and an arrow points to it with the label 'Same position of other samples within selected PicDB database'. Other controls include 'Average' histograms, 'PicDB' options (Rainbow/Grayscale), 'Brightness' slider, and 'Filter'/'Show' buttons.

Base	1	3	5	9	11	13	15	17	19	21	23	24
1	A	G	C	C	G	A	G	G	T	A	T	G
2	A	G	G	C	C	T	-	C	A	T	A	C
3	G	G	C	G	G	G	G	G	G	G	G	C
4	C	C	G	C	C	C	C	C	C	C	C	G
5	G	G	G	C	T	C	C	G	C	C	A	C
6	C	C	C	G	A	G	G	C	C	G	T	G
7	C	T	C	G	G	C	G	A	C	G	G	G
8	G	A	G	C	C	G	C	T	G	-	C	G
9	T	C	A	A	G	C	A	C	C	G	G	C
10	A	G	T	T	C	G	T	C	G	G	C	G
11	-	C	C	C	A	G	C	G	C	A	C	G
12	G	G	G	G	C	-	G	C	G	T	G	G
13	C	A	T	G	G	G	C	G	T	C	G	T
14	G	T	A	C	C	C	G	C	A	G	C	A
15	C	G	A	G	G	A	C	C	C	T	T	C
16	-	C	T	C	C	T	G	G	G	A	A	G
17	G	C	G	G	G	-	T	C	C	G	G	T

Base calls table

Reference and Notes window

The image fragment of selected cell in each channel

Same position of other samples within selected PicDB database

Figure 13. The results window

2.6.1 Identifying and Accepting Signal Values for Each Channel

Click the **A**, **C**, **G** or **T** menu to view signal values for each channel. There is a separate table for each channel.

The light blue cells show a possibility of an artifact: a signal has been identified, but it is not confirmed by a repeat of the same spot. This may happen if the same oligonucleotide is printed on the slide in duplicate (See also section 2.7.2).

As a tracking feature, already inspected cells have a darker background.

To identify and accept signal values do the following:

- Click a cell to select it or press the **space** key on the keyboard to see the images corresponding to particular signal.
- Verify signals visually (See Figure 15.).
- To select the next light blue cell press the **Z** key

Genorama® BaseCaller™ and **PicDB™** permit for **filtering against known false-positive signals** in microarray.

To use the filter do as follows:

- Open or create a new **Notes file**. Add **excl N** or **exclude N** to the end of the note for the particular microarray position. N is the image (A, G, C or T) where quantitation is not required.

2.6.2 Genotyping Results in Base Calls Table

Click the **Base Calls** menu of the results window (See Figure 13.) to view base calls table. Base calls table is the main tool for sequencing and genotyping results according to the Arrayed Primer Extension (APEX) microarray.

Light blue cells in the table show that this position needs to be verified visually (See Figure 13.).

Already inspected cells have a darker background.

Exclamation mark (!) is used if the given base and the Genorama® reference file with expected base calls do not match (See section 2.6.5).

To edit base calls table do the following:

- **Click** a cell to select it or press the space key on the keyboard to see the images corresponding to particular base call.
- Press the **Enter** key on the keyboard to correct values.

To compare the results with other samples:

- Click to the button **Open PicDB**
- Select the position in base calls table
- Click to the button **Show**.

You can reset all the changes you have made in base calls table or you can load saved base calls table.

2.6.3 Identifying Signals in Detailed View Window

To identify Signals in Detailed View Window of selected cell:

- Double-click on any of the four signal windows every pixel is one block and its height depends on signal intensity.

In detailed view you can view image with different palette and brightness values. Different image palettes are for better visual identification and do not affect the results.

The Zoom tool lets you view the signal from front and back view or adjust the scale for signal intensities.

Use those tools to identify visually complex or faint signals.

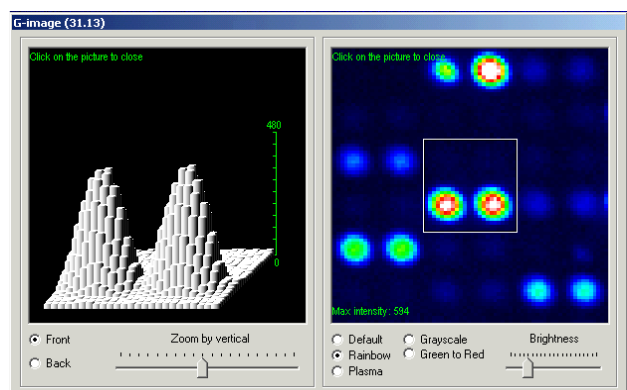


Figure 14. Detailed view of selected cell

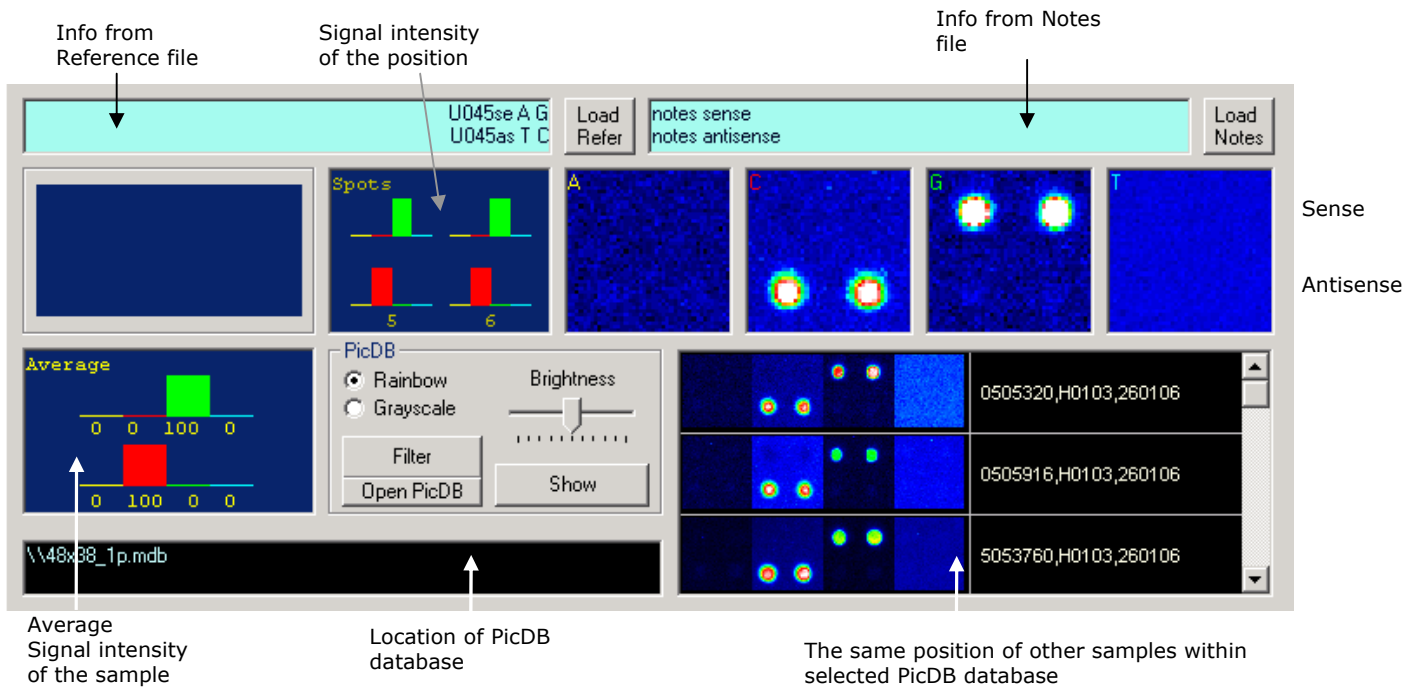


Figure 15. Part of results window.

2.6.4 Using Notes

Use Notes tool to store comments or performance of particular array cells such as possible unspecific signals.

To edit notes table do one of the following:

- Click the **Notes** menu to open notes table.
- **Click** a cell to select it or press the **space** key on the keyboard.
- Press the **Enter** key on the keyboard to enter new value.
- **Click** the **Notes** window on the lower left corner of the Results window and enter new value.

To undo changes in notes table do one of the following:

- Choose **Reset notes** from **Settings** menu to undo all changes you have made.
- To open saved notes table click to the button **Load Notes**.

Notes file permits for **filtering against known false-positive signals** in microarray (see also section 2.6.1).

*Note: The notes made will be saved with **grn** file extension*

2.6.5 Using Reference

To edit reference table do the following:

- Click Reference menu to open reference table.
- Click a cell to select.
- Press the Enter key on the keyboard to enter new value.
- Two last symbols if these consist of A, C, G or T, will be used for comparing with the sample basecall. The other symbols may indicate the gene, base position in the sequence, SNP ID and DNA strand (se and s - sense, as - antisense), for example:

TP53 12139se CG

To undo changes in reference table do one of the following:

- Choose Reset reference from Settings menu to undo all changes you have made.
- Choose Reference table from Load menu to open saved reference table.

*Note: The reference file will be saved with **grf** file extension*

2.7 Signal Normalization and Results Window Settings

By default, signals are compared according to their actual values from microarray quantification (real values). However, the scale and intensity distribution of signals may differ between the different microarray images corresponding to different fluorescence channels. These differences are reduced by different normalization methods.

To specify signal normalization method do the following:

- Click the **Normalization** menu on the Results window (See Figure 13.).

By the signal maximum value The few most intense signals in each image will be used for linear normalization.

The software includes two empirical data normalization algorithms:

By weakest nucleotide 100 most intense signals from each of the images are selected as a reference for comparison. The average values for this set of signals are compared and all signals from three images (except the one with the smallest average value) will be divided with the variable from the comparison of 100 most intense signals.

By distribution of signals This assumes that the signal intensity histograms must be equal between different images. All signals from each image are ranged (1st, 2nd, 3rd, etc.). Signals with the same number in the sequence are compared and the more intense signal values adjusted to the level of the signal value from the weakest image (e.g the second strongest signal from A, C and T images must have the same value as the second strongest from G image, etc.).

2.7.1 Visualizing Results With Spectra and Graphs

Click the **Chart** menu on the Results window (See Figure 13.)

To visualize results click one of the following buttons:

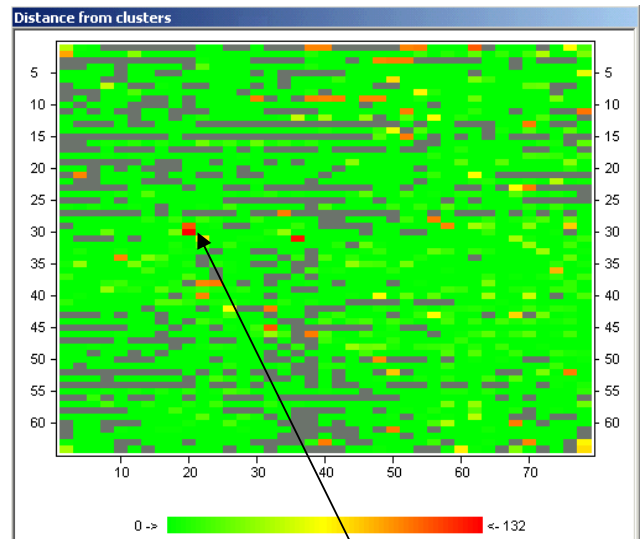
Histogram of intensities Use this tool to see the number of signals corresponding to different signal intensities.

Histogram of distances Use this tool to view distribution of distance measure used for comparison with wild-type reference DNA.

Number of clusters Use this tool to view the number of data clusters per microarray position (per given APEX oligonucleotide). The graph illustrates the reproducibility of signal patterns with a wild-type reference DNA if this is used for comparison with the analyzed sample.

Distance from clusters Use this tool to visualize distances (differences) from the reference dataset (See Figure 16.). The positions with zero distance have a perfect match with the analyzed sample. The bigger the value of distance measure is the bigger is the probability of mutation.

Clarity of signals Use this tool to show positions with multiple secondary signals in case of otherwise homozygous samples (summarizes signal values between 0 and 100%). The graph is useful when determining the reaction specificity, but is not informative with a chip showing many heterozygous positions. The scale is shown



Mutation site differs from signal data obtained with the wild-type reference sample

below the graph. Dark grey areas show missing signals on the chip.

Figure 16. The distance from clusters window

2.7.2 Specifying Base Calls Table Options

Use the base calls table options to specify when cells are shown with a light blue background. The blue cells indicate the possibility of an artifact in microarray images (according to the selectable criteria in **Options** → **Blue Cells** panel) and prompt user to analyze the address manually.

To assign Blue Cells in BaseCaller™ main window and results window:

- In BaseCaller™ main window choose **Options** from **Tools** menu
- In Results window choose **Blue Cells** from **Settings** menu
- **Single signals above the minimum level of secondary signals** Use this option to check all cells where both recurrent signals and weaker single signals are found.
- **Possible errors or artefacts (marked as '!')** Use this option to check all cells, which do not match the reference table and may indicate mutations.
- **Missing signals (marked as '.')** Use this option to check the cells where no signals are identified.
- **Exceeding distance from clusters** Use this option to check all cells, which do not match the clustered signal patterns from the reference sample.
- **Sense/anti-sense non-conformities** Use this option to check all cells where the signals from sense strand do not match the signals from antisense strand. *

* *Note: It is assumed that both strands give signals complementary to each other. This is the situation with typical oligonucleotides for APEX, but not with allele specific oligonucleotides (ASO).*

2.8 Saving and Exporting Results

Genorama® lets you save and export signal and base calls tables, reference and notes table.

To save signal tables do the following:

- Choose **Four tables: A, C, G, T** from **Save** menu

To save base calls table do the following:

- Choose **Current table** from **Save** menu with base calls window active

To save reference table do the following:

- Click the **Reference** menu.
- Choose **Current table** from **Save** menu.

To save notes table do the following:

- Choose **Notes table** from **Save** menu
- Save **Current table** as **Notes table**

To save full signal statistics do the following:

- Choose **Save full signal statistics** from **Tools** menu of BaseCaller™ **Main** window. **Save** window appears. Select the folder, appropriate filename and proceed with signal scanning.

To export signal tables do one of the following:

- Choose **Current table** from **Excel** menu.
- Choose **Signal tables as column** from **Excel** menu.

To export base call table do one of the following:

- Choose **Current table** from **Excel** menu.
- Choose **Base call table as string** from **Excel** menu.
- Choose **Base call table as column** from **Excel** menu.

To export reference table do the following:

- Click the **Reference** menu.
- Choose **Current table** from **Excel** menu.

To export notes table do the following:

- Click the **Notes** menu.
- Choose **Current table** from **Excel** menu.

Note: If untracked blue cells are exported, question marks appear in the cells.

2.8.1 About File Formats for Signal values, Basecalls and Notes

BaseCaller™ results are presented in table format and can be saved in many formats:

- Signal intensity tables (Genorama® results table, *.grs; full signal statistics table *.txt)
- Text table (Genorama® base calls, *.grb)
- Table of notes (Genorama® notes, *.grn).

All the results can be exported to MS Excel in various selected formats.

Full signal statistics table (See Figure 17.) contains information on image, address, signal intensity value, signal pixels (S_mean, min, max), local background pixels (B_mean, min, max), signal area in pixels (S_area), diagnostic comment if the signal is not found, Notes and Reference file.

For information on diagnostic comments, please contact Asper tech support.

Examples of the comments: W3-very low intensity of the possible signal, C3-very low number of possible signal pixels.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Image	Address	Intensity	S_mean	S_min	S_max	B_mean	B_min	B_max	S_area	Comment	Notes_file	Ref_file
2	A	1.1	8	15	11	22	5	1	10	20			N
3	A	1.2	10	17	9	25	6	1	12	25			
4	A	1.3	15	23	15	38	6	1	15	25			A
5	A	1.4	18	27	15	45	7	1	18	26			
6	A	1.5	2	7	5	9	4	0	8	25			G
7	A	1.6	1	6	4	9	4	1	8	24	W3		
8	A	1.7	1	6	5	9	4	0	7	8	W3		11687sA
9	A	1.8	0	0	0	0	0	0	0	0	C5		

Figure 17. Full signal statistics table.

3 PicDB™

3.1 Working with PicDB™ Image Database

You can create the database of APEX images corresponding to each cell on the grid by using the BaseCaller™ program. PicDB™ lets you to compare all the array positions at all images. The images can be analyzed visually or with different quantitative formats.

Note: According to signal intensities from four different channels, all images corresponding to same address are shown in two scatter plots, for sense and antisense strands.

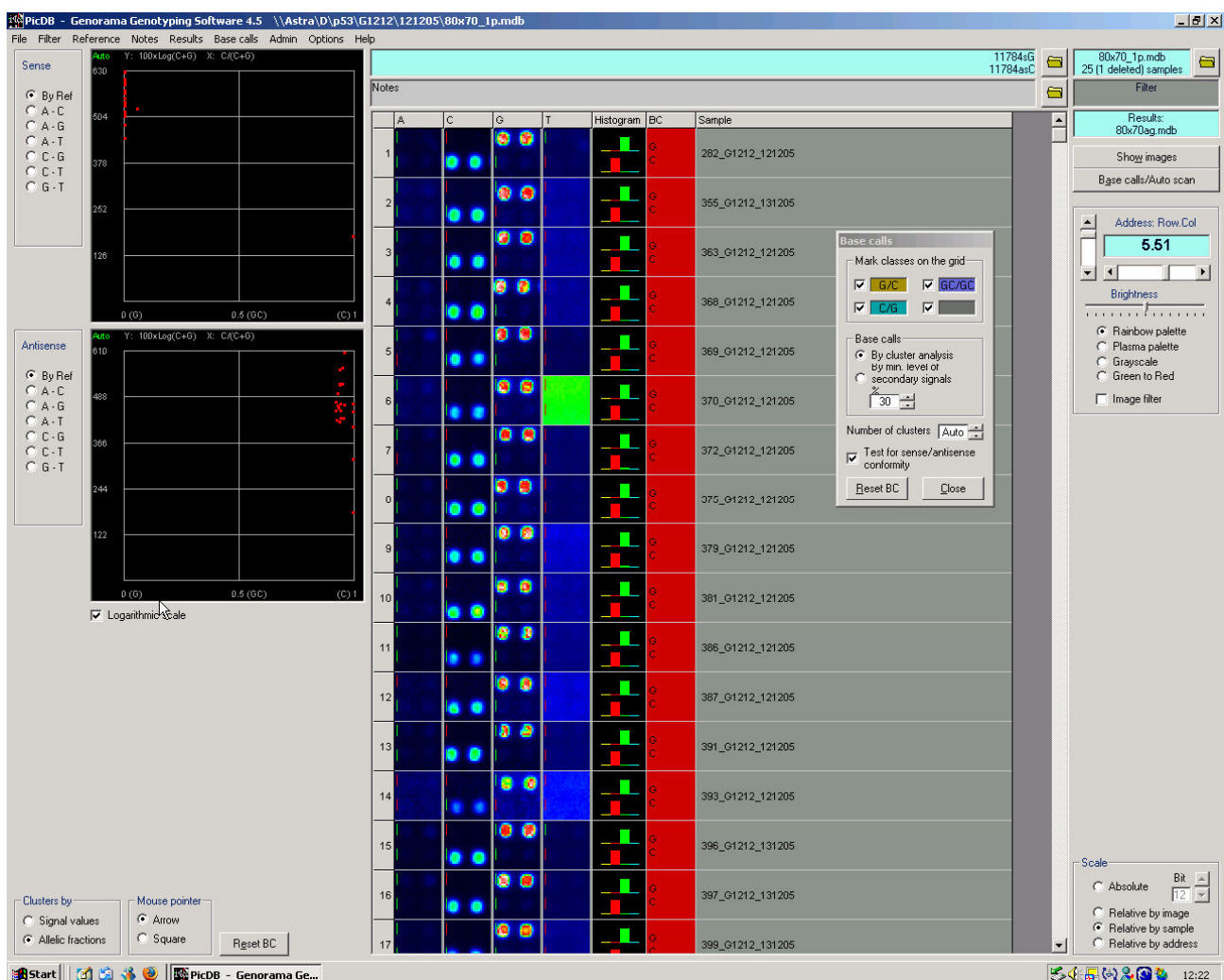


Figure 18. PicDB™ image database analysis program main and base calls windows

3.1.1 Opening Image Database

To open image database:

- Choose **Load image database** from File menu.

Image databases are automatically named according to their grid dimension values. For example if your grid has 20 horizontal and six vertical cells, the image database name is **20x6_1.mdb**. Databases exceeding 500MB will be automatically split by the software (20x6_1.mdb, 20x6_2.mdb, etc.). User will still be able to open all the data simultaneously by opening just the first file 20x6_1.mdb. (See also section 2.4)

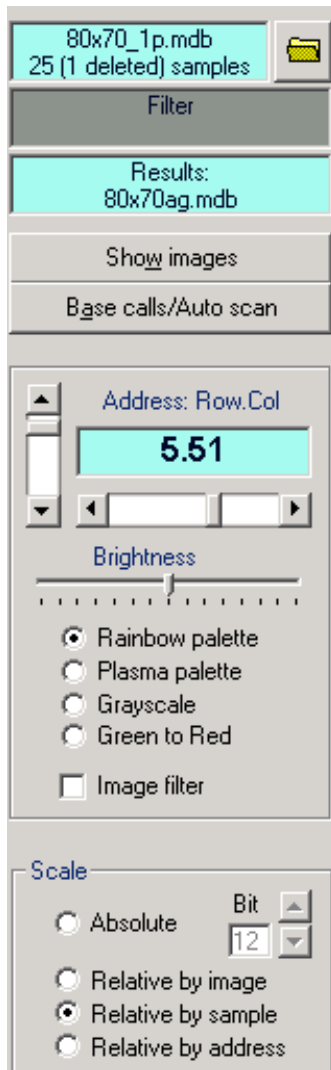


Figure 19. PicDB™ main window toolbar.

3.1.2 Determining Image Address

To specify sample position from the database:

- Drag the **Address: Row. Col.** sliders

Vertical slider changes row address and horizontal slider changes column address.

Z and **X** keys set focus on vertical and horizontal sliders and the address may be changed by pressing respective **arrow key**.

3.1.3 Adjusting Image Brightness

Using brightness command is the simplest way to adjust appearance of your samples.

Click the **Show/Scan images** button on PicDB™ main window toolbar (See Figure 19.) to view image database in determined position. All positions from different images are visualised and automatically scanned. Also, there are histograms and automatic base calls.

To adjust image brightness do the following:

- Drag the **Brightness** slider right to increase samples brightness level.
- Drag the **Brightness** slider left to decrease samples brightness level.
- Click the **Show/Scan images** button to view samples with new brightness level.

3.1.4 Choosing Image Palette

PicDB™ lets you view images with **Rainbow**, **Plasma** or **Grayscale** palette (See also Figure 4.). Different image palettes are for better visual identification and do not affect the results.

To change image palette do the following:

- Click the **Rainbow**, **Plasma** or **Grayscale** radio button.
- Click the **Show/Scan images** button to view samples with new image palette.

3.1.5 Scaling Images

Use this option to specify the visualized scale of the microarray images.

Absolute This scale corresponds to the actual scale of TIFF images. Use this option to see if any saturated signals exist.

Relative by image This scale shows all signals independent on their intensities. This is the recommended visualization option for routine analysis.

Relative by sample This scale corresponds to the minimum and maximum intensities of the whole sample. Use this option to compare the signal intensities between A, C, G and T images.

Relative by address This scale corresponds to the minimum and maximum intensities of the whole image database. Use this option to compare the signal intensities between different samples.

3.1.6 Changing Image Scanning Options

To change scanning options do the following:

- Click **Options** menu on menu bar, the options table appears. Mostly the options are the same as in BaseCaller™ (See section 2.5.1), there are few PicDB™ specific scanning options:

Show red-green corners use this option to visualize found signals. All the green ones are found signals and red ones are not.

Recurrent signals only This option affects the results if there are some missing signals. If one of the two recurrent signals is missing, then it won't be considered as true signal. This option affects signal intensities, histograms, basecalls and graphs.

Min. level of secondary signals for basecalling Use this option to check all positions where both recurrent signals and weaker single signals are found.

Local background subtraction - Use this option to subtract the value of the background around the spot from the integrated volume of pixel intensities used for calculation of the signal intensity value.

Sensitivity - Use a sensitivity threshold to eliminate low intensity signals
0% - only signals with high intensity are counted
100% - all found signals are counted and quantified.

To view samples with new image scaling and adjusted scanning options:

- Click the **Show/Scan images** button.

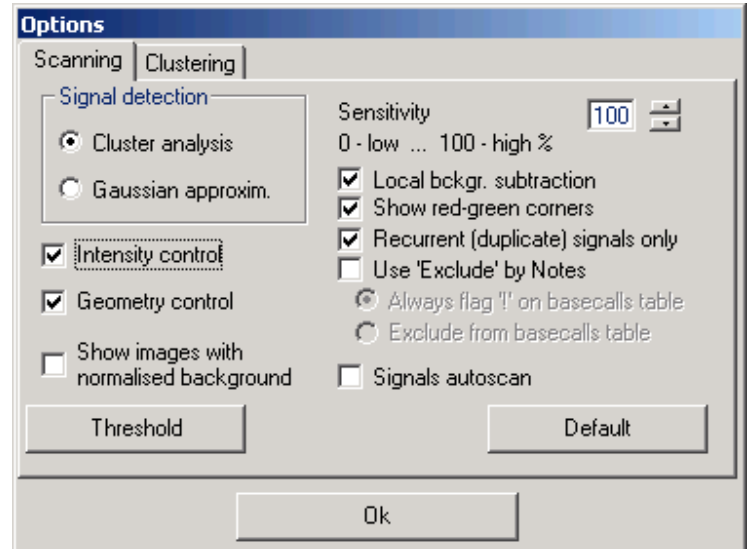


Figure 20. The Image scanning options window in PicDB™.

3.1.7 Using Sample Filter

PicDB™ lets you filter your samples by name or against name.

To apply sample filter do the following:

- Click the **Filter** menu. The filter window (See Figure 21.)
- Enter the sample name(s) and click **OK**. Samples with a given phrase in the sample name will be shown or hidden.

To select particular samples for analysis:

- Click **Selected samples** button. The selected samples window (See Figure 22.) appears.
- Select the number of sample to be viewed in main window.

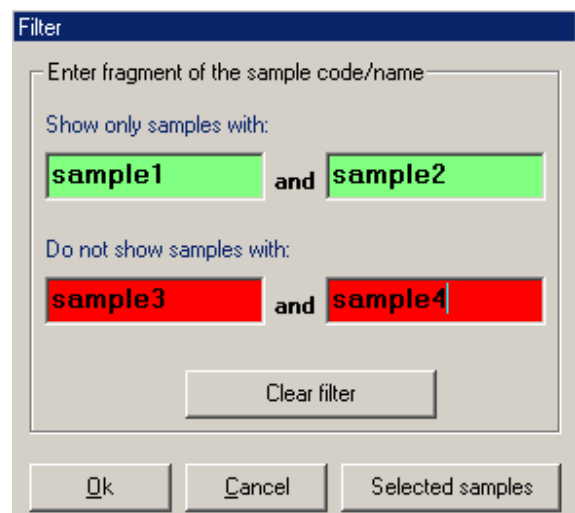


Figure 21. The filter window

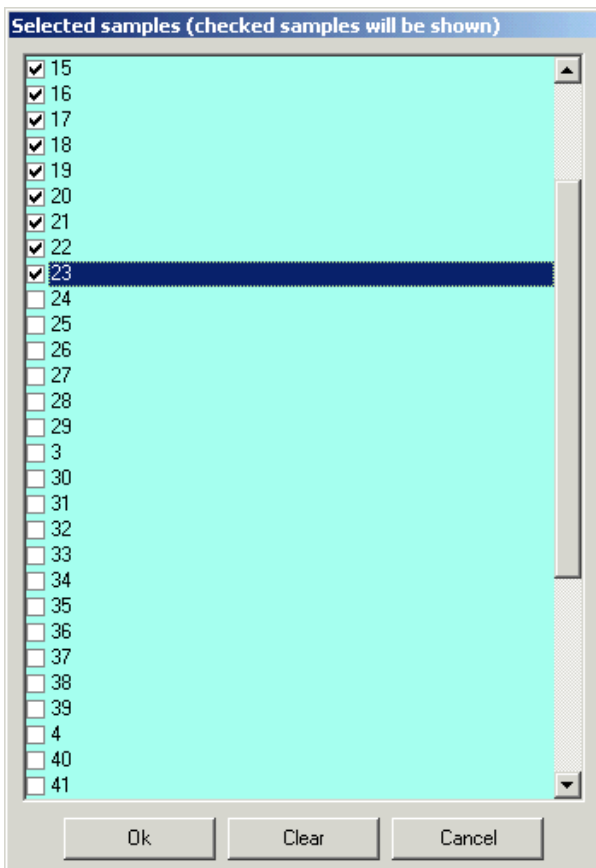
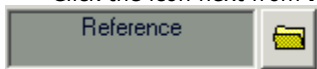


Figure 22. The selected samples window.

3.1.8 Using Reference

To select the appropriate reference file:

- Click the icon next from **Reference** window



- Drag the **Address: Row. Col. Sliders** to select the microarray position for analysis.
- Reference information for the same position will be shown.

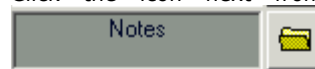
To create a new reference file:

- Select **New** from **Reference menu**.

3.1.9 Using Notes

To select the appropriate notes file:

- Click the icon next from **Notes** window



Use the Notes tool to see saved comments or notes on performance of particular array positions such as possible unspecific signals.

To create a new notes file:

- Select **New** from **Notes menu**.

3.2 Grouping Samples in Graph Window

The Graph tool lets you group the samples. On Scatter-plot all dots can be selected and analyzed separately. The white dots are not yet analyzed, the green dots are selected and the red dots are already edited or saved.

3.2.1 Specifying Graph Drawing Options

Logarithmic scale Deselect the logarithmic checkbox to see signal values in linear scale.

Clusters by:

Signal values Use this option to set two specified images (e. g. A and G) to correspond to X and Y-axis.

Allelic fractions Use this option to set X axis to show the summarized signals from two specified images and Y axis to show the distribution of signals according to their allelic fractions.

To select samples with the Graph tool do the following:

- Select **Arrow** or **Square** radio button
- **Click** the dots you want to select in Sense or Antisense window.

To select samples from Base Calls (BC) column do the following:

- Press **Shift** key and **click** on the value you want to select in the BC column.
- Alternatively, select all basecalls by pressing **Shift** key and **clicking by mouse** left button with the pointer on the BC column label. This selects only the raw, nonsaved data (with light grey background).

Histogram	BC	Sample
	G C	1-21,001
	G C	1-21,002

To deselect samples with Graph tool do the following:

- Select **Arrow** or **Square** radio button
- Press **Shift** key and click the dots you want to deselect in the Sense or the Antisense window.

To deselect samples from Base Calls (BC) column do the following:

- Press **Shift** key and **click** on the value you want to deselect in the BC column.

To view where the certain sample is located on graph do the following:

- Click on the sample number

To reset graph do the following:

- Click the Reset button.

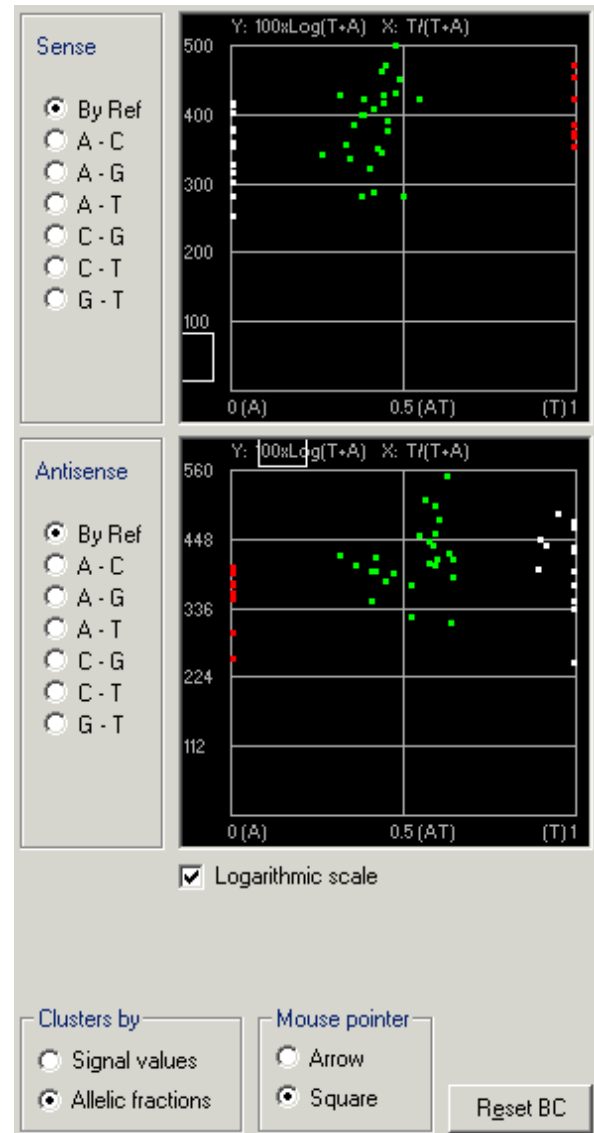
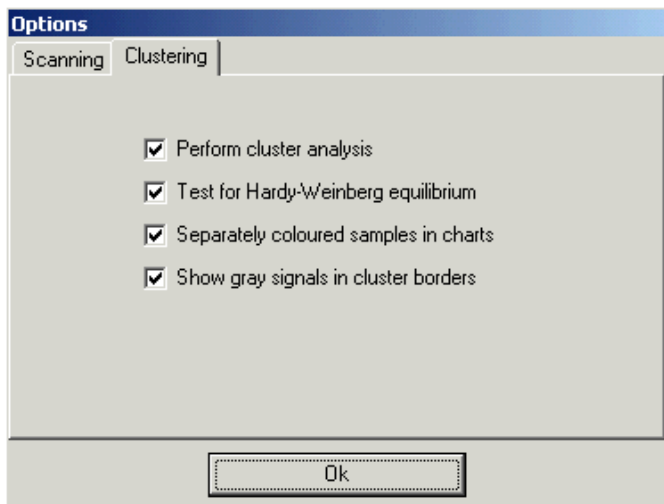


Figure 23. Part of PicDB™ main window

3.2.2 Cluster Analysis of Genotyping Results

PicDB™ has a built-in clustering tool for the genotyping results. The tool is mostly optimized for single nucleotide polymorphism genotyping



To use the clustering tool for visual indications of the assumed genotype clusters:

- Open the **Options panel**.
- Select **Perform cluster analysis**.
- The **colouring options** in charts include a vertical ribbon or small circles in the background of the data for each sample
- **Gray signals in cluster borders** will eliminate errors in rare cases, where the genotype clusters will overlap in result of the automated analysis from both strands (recommended)

Figure 24. PicDB™ cluster analysis options for basecalling and visualization in charts

3.3 Working with Samples

3.3.1 Identifying Signals in Detailed View

Double-click on any of the sample to open the detailed view of selected cell. Every pixel is one block and its height depends on signal intensity.

The Zoom tool lets you scale signal intensities. Use it to identify complex or faint signals visually.

3.3.2 Editing Base Call Values

Click the sample base call (BC) field and enter the new value.

To set the same base call values for all selected samples:

- Click the **Same base call for all selected images** checkbox (See also section 3.2.1). Note: The changes will also affect all basecalls from cluster analysis, i.e. **BC fields with identical background** colour.

To use cluster analysis for genotyping:

- Click on Base calls/Auto scan button or press Alt+A.
- Select whether BC field will be formed of cluster analysis results or by minimum level of secondary signals for each primer site.
- Select the number of clusters required (Auto, 3, 2, 1).
- Testing for **Sense/antisense conformity** provides additional precision for automated basecalling

Note: to keep the selected options, close Base calls window

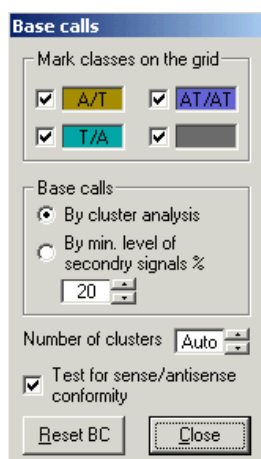


Figure 25. PicDB™ Base calls window

	A	C	G	T	Histogra	BC	Sample
24						G C	26
25						CG CG	27
26						C G	28
27						C G	29
28						C G	30
29						C G	31
30						C G	32
31						C G	33
32						C G	34
33						CG CG	35
34						CG CG	36
35						CG CG	37
36						G C	38
37						G C	39
38						G C	40
39						G C	41
40						G C	42
41						CG CG	43
42						CG CG	44

Figure 26. Part of PicDB™ main window

3.3.3 Changing Sample Name

PicDB™ lets you change sample names you set in BaseCaller™ (See section 2.4).

To change sample name do the following:

- Choose **Admin** menu from Menu Bar of **PicDB™** image database analysis program main window (See Figure 18.) . The **Admin – rename, delete and restore samples** window appears.
- Select the sample name from the list, the name appears in the black area.
- Click **Rename** and type new name.
- Click **Save**.

3.3.4 Deleting and Restoring samples

To delete samples do the following:

- Choose **Admin** menu from Menu Bar of **PicDB™** image database analysis program main window (See Figure 18.) . The **Admin – rename, delete and restore samples** window appears.
- Select the sample name from the list, the name appears to the black area.
- Click **Delete**.

Deleted samples remain in the database, but are not visible in PicDB™ analysis window.

Deleted samples can be easily restored.

To restore deleted samples do the following:

- Choose **Admin** menu from Menu Bar of **PicDB™** image database analysis program main window (See Figure 18.) . The **Admin – rename, delete and restore samples** window appears.
- Select the sample name (with ***DEL*** in the beginning) from the list, the name appears in the black area.
- Click **Restore** to restore deleted samples.

3.3.5 Listing Samples

To see the list of samples do the following:

- Choose **Admin** menu from Menu Bar of **PicDB™** image database analysis program main window (See Figure 18.) . The **Admin – rename, delete and restore samples** window appears.

3.4 Saving and Exporting Results

3.4.1 Saving Results

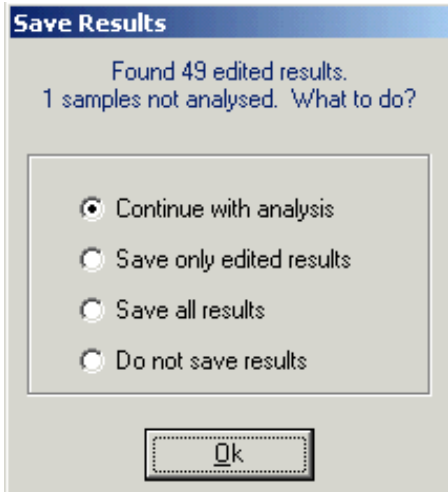


Figure 27. The save results window.

3.4.2 Visualizing Results

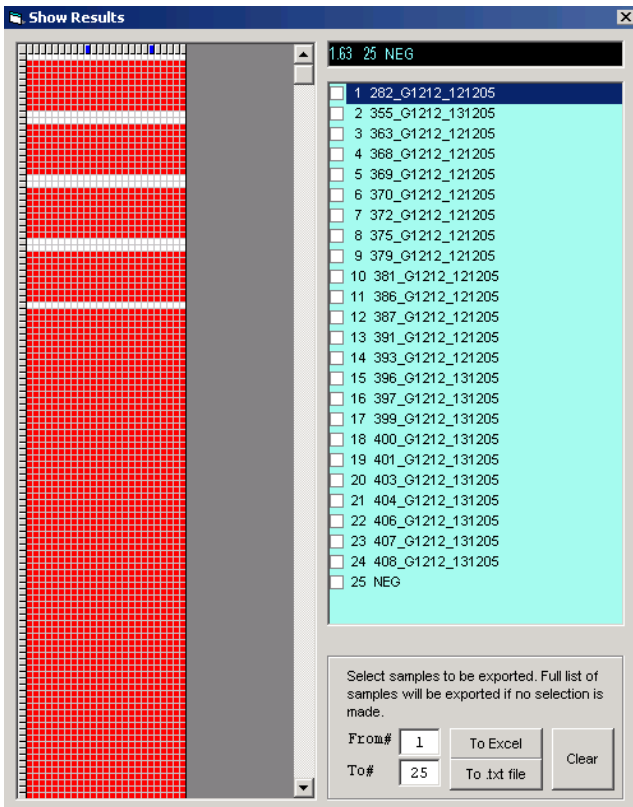


Figure 28. The **Show results** window.

To save results do the following:

- Choose **Save results** from **Results** menu. The save results window (See Figure 27.) appears.

Continue with analysis Choose this option to close dialog box without saving. This will keep your selection and you can continue with analysis of the same microarray position.

Save edited results Choose this option to save the edited results. The edited results consist of cells with **any coloured background from analysis**, except for the default light gray background for raw base calls

Save all results – choose this to save the analyzed position in the whole database

Do not save results - Choose this option to cancel saving and go to the next position.

To visualize results do the following:

- Choose **Results** from menu bar.
- Choose **Show Results/Export to Excel**.
- The show results window (See Figure 28.) appears.

The **Show results** tool lets you see, which positions and samples are already analyzed and saved. Red cells mark the saved positions.

3.4.3 Exporting Results to Excel

All the results can be exported to MS Excel in various selected formats.

To export results to Excel do the following:

- Click on the button **To Excel** to export the results to MS Excel
- Or click on the button **To txt.file** to export the results to Notepad.

3.4.4 About File Formats

PicDB™ Results are automatically saved in **Agenda** file (...**ag.mdb**) in the same folder with the Image Database analyzed.

4 Genorama® Converter™

4.1 Converting Text Table to Genorama Reference Table

Genorama® Converter™ utility lets you convert a tab delimited text table into a Genorama 4.5 reference table or 20-space blocks for Genorama 4.2 or older versions. The Excel table has to be made in the similar layout as the final grid on the slide (See Figure 29.).

To convert the Excel file onto Genorama® Converter™ reference table, save the table as tab delimited text first.

To convert tab delimited text table into a Genorama reference table:

- Open Genorama® Converter™
- Click **File** on menu bar to load txt file
- If you use Genorama 4.5 choose **Save in new standard** option and if you use Genorama 4.2 or older choose **Save in old standard** option (See Figure 30).
- Click to button **Save** and choose the target folder.

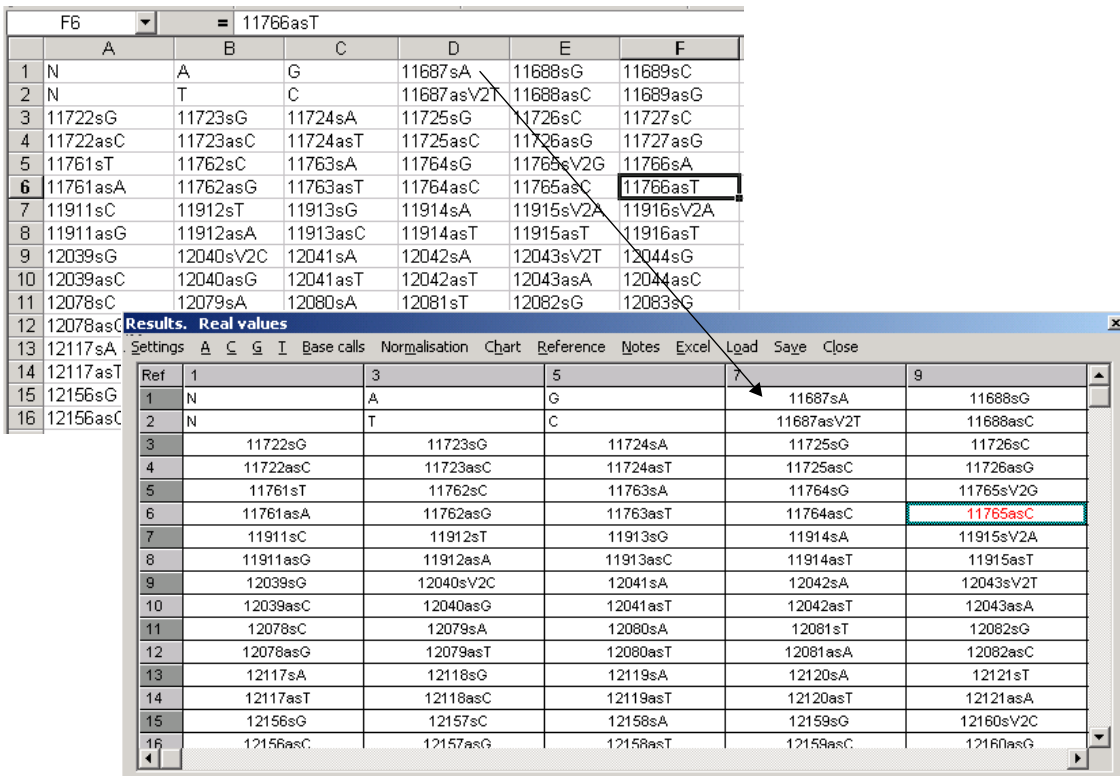


Figure 29. Similar layouts of Reference files in MS Excel and Genorama® BaseCaller™.

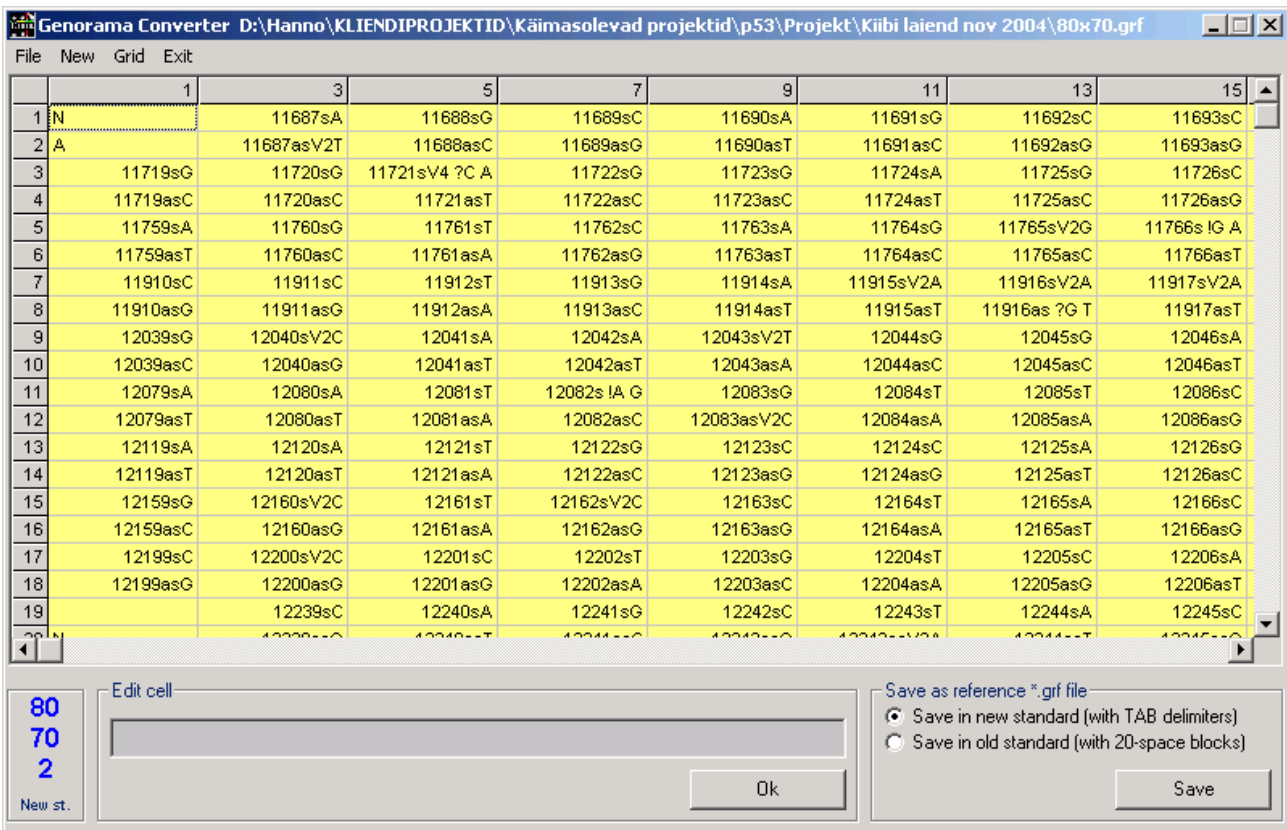


Figure 30. Genorama® Converter™ main window.

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