X29. Sample Tracking

This portion of the OmniGrid software allows the user to track where samples have been printed on the slides. The software combines information (supplied by the user) about the sample plates with the chosen method's parameters to provide a deconvoluted file that can be used by scanner software programs.

> ***Note:*** **The terms "Sample tracking" and "deconvolution" will be used interchangably throughout this chapter.**

As with the Run Parameters menus, the Sample Tracking menus are different depending on the configuration of the OmniGrid.

The two situations are:

- **If customers are running the sample tracking software for methods that were printed without the use of the server arm, they should refer to Sections 29.1, 29.3 and 29.4 of this chapter.**
- **Customers using the sample tracking software for methods and samples printed with the server arm should refer to Sections 29.2, 29.3, 29.4 from this chapter. These customers also need to make sure that the server arm control box (*See 18.2 Set-up of the server arm on page 62*) is connected properly and has been powered *ON* prior to entering the Gridder software to do deconvolutions.** The software will automatically detect that the plate loader is on and will open the appropriate Sample Tracking menu. The user does not need to do anything to facilitate this.

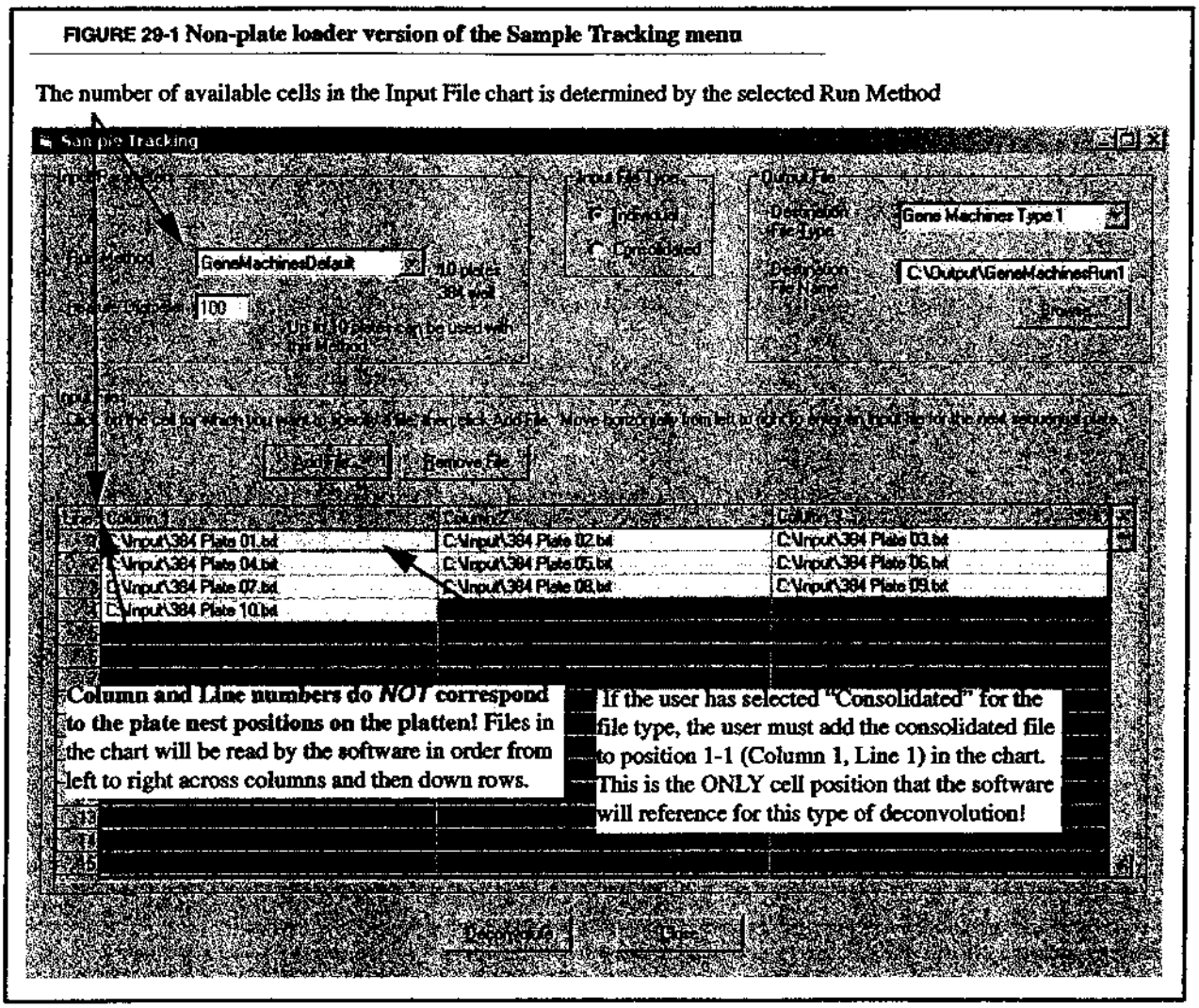
29.1 SAMPLE TRACKING: NON-PLATE LOADER VERSION

1. **Click on the SAMPLE TRACKING button in the Select Gridder Operation menu.**
   This will open the Deconvolution screen (see Figure 29-1).

29.1.1 Input parameters

1. **From the first pull-down menu, choose the Run Method name that was used for the printing run.**
   Remember that if the method has been changed since the samples were printed onto the slides, the deconvolution may not be done correctly! **While it is not necessary to do the deconvolution immediately after completing the printing run, it is recommended. This will help avoid any confusion.** (The output files take up very little memory and can easily be deleted if they are not needed.)
2. **If the output file type is set to Axon® GenePix™ ArrayList (see Section 29.1.4.5), the user will have the option to specify the approximate spot size (in microns) next to Feature Diameter.**
   The scanner software for this file type will set use this information to set up the spot grid that has the proper array dimensions and spot sizes. (This grid is superimposed over the array images in the scanner software.)

FIGURE 29-1 Non-plate loader version of the Sample Tracking menu



29.1.2 Input file type

The software accepts two input file types, *Individual* and *Consolidated.* If there are multiple input files, they must all be of the same type. This section describes the required format for these two file types as well as the best method for creating these input files.

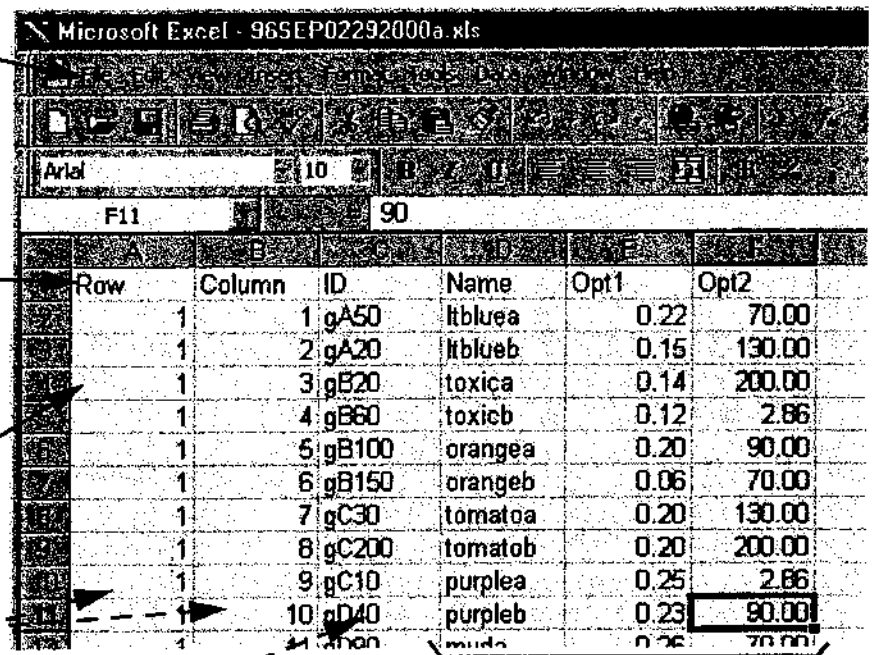
29.1.2.1 Individual

This file type will require that the information for each sample plate is in a separate file. The user will add each sample plate file to the Plate File List in the order in which the it was printed during the run.

Several formatting rules must be followed when creating an Individual input file:

- **It must be a tab delimited text file (see Figure 29-3).**
  The simplest way to create this file type is to enter the required plate information into a Microsoft Excel file (see Figure 29-2) and then save the file as a tab delimited text file.

FIGURE 29-2 Example Excel file: Individual format



Once the information has been entered correctly, select the Save As option (from the File menu) and save the file as a ***Tab Delimited Text File***.

The sample tracking software requires a one-line header. (Only the first cell must be labeled -- the "row" label in this figure.)

The Row values can be numbers or letters

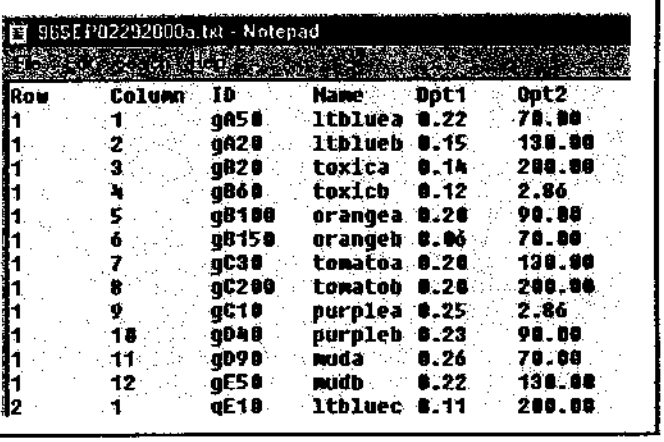
The software detects the end of the plates using the Row and Column values of 8 and 12 (for 96-well plates) and 16 and 24 (for 384-well plates). (Not visible here.)

The last cell under the ID column must have a value entered. (Not visible here.)

The Name, Opt1 and Opt2 fields are optional. Not all of the output file types will pass this information through.

- **It must include a Row number or letter, Column number, and an ID name or number for each sample.**
  In the example shown in Figure 29-2, the rows are listed as numbers. The sample tracking software will also accept letters for the Row entries. This latter option may be less confusing than converting the row numbers to letters (eg. 'A' is 1, 'B' is 2, etc.)
- **It must include a one-line header row.**
  It is important that this header is one, and only one, line/row! While it is not necessary to have entries in the cells above each column of information, the sample tracking software will require the first cell in the header to have a label.
- **If certain destination file types are chosen in the Sample Tracking window, additional information can be incorporated from the input file.**
  The two GeneMachines output file types can each pass through a Name and two more optional pieces

FIGURE 29-3 Tab delimited input file (Individual type)



of information for each sample (labeled Opt1 and Opt2 in Figures 29-2 and 29-3). Axon® GenePix™ ArrayList file type incorporates a Name.

- **To recognize the end of a plate and the plate type (96- or 384-well), the software will look for certain Row and Column values.**
  The input file ***MUST*** have the proper values in the last Row and Column cells, or the software will not process the file! (Row 8, or H, and Column 12 for a 96-well plate or Row 16, or P and Column 24 for a 384-well plate).
- **To recognize the end of the file, the software will look for an entry in the last cell under the ID column.**
  Even if there is no sample in this position, the ID field ***NEEDS*** to have an entry (such as "none"). (Remember that this will be next to a Row 8, or H, and Column 12 for a 96-well plate, or Row 16, or P and Column for a 384-well plate.)

29.1.2.2 Consolidated

The consolidated file type will require that all of the sample plate information is located in a single file in the order in which the plates were printed.

Several formatting rules must be followed when creating a Consolidated input file:

- **The format for a Consolidated file is the same as for an Individual file, except that it requires a Plate number column (see Figure 29-4).**
  The plate numbers indicate the order in which the plates were printed. This Plate column must be the first one (i.e. left-most)!
- **As with the Individual files, the software will look for Row 8 or H, and Column 12 for a 96-well plate and Row 16 or P, and Column 24 entries for a 384-well plate to recognize the end of each plate.**
- **The plate information must be in the same order as the plates were printed in the method.**

FIGURE 29-4 Example Excel file: Consolidated format

For the consolidated format, an additional Plate column must be included before the others. It is also important that the plates are listed in the same order as used for the printing run.

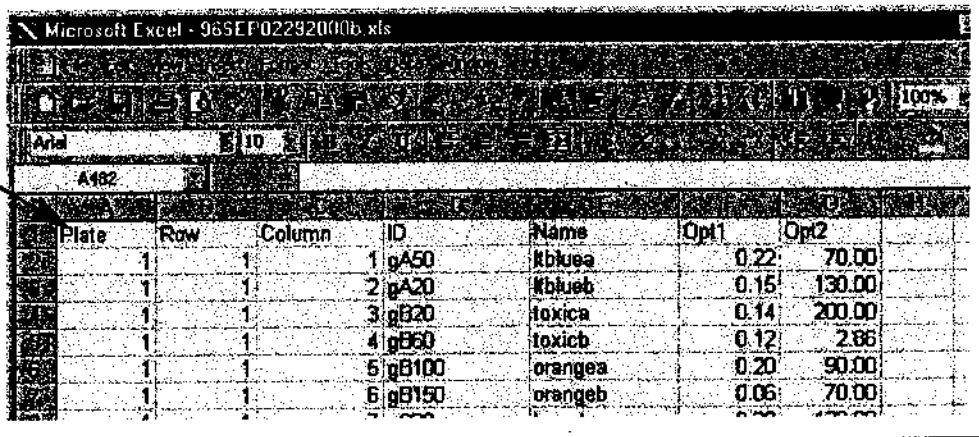


	Plate	Row	Column	ID	Name	Opt1	Opt2
2	1	1	1	gA50	ltblue a	0.22	70.00
3	1	1	2	gA20	ltblue b	0.15	130.00
4	1	1	3	gB20	toxic a	0.14	200.00
5	1	1	4	gB60	toxic b	0.12	2.86
6	1	1	5	gB100	orange a	0.20	90.00
7	1	1	6	gB150	orange b	0.06	70.00

29.1.3 Input file chart

Once a run method has been selected, the Input File chart will display cells for the total (max) number of sample plates in the run. (Unused cells are greyed-out.)

> ***Note:*** **Keep in mind that the Column numbers in this Input File chart *DO NOT* correspond to plate positions on the OmniGrid platten. This can be confusing at first!**

1. **Make sure that the Input Plate Type has been selected.**
2. **Click on the Column 1, Line 1 cell (position 1-1) in the Input File chart to select it.**
3. **Click the ADD button.**
4. **The software will open a browser window that will allow the user to select a file. Click OK in the browser window once the correct input file has been selected.**
5. **If the input files are the Individual type, continue adding to the chart the sample plate files that were used in the run.**
   The second file goes into the Column 2, Line 1 (or, position 2-1); the third file into Column 3, Line 1 (position 3-1); the fourth into Column 1, Line2 (position 1-2); and so on.

   Remember that if a plate is used multiple times during a run, it will be necessary to add that plate file to the Input File chart each time that it is used during the run. For example, assume that the order in which a set of 3 plates was used is as follows, Plate A, Plate B, Plate C, Plate A, Plate C. The Input Chart file should be set up as follows:

Line	Column 1	Column 2	Column 3
1	PlateA.txt	PlateB.txt	PlateC.txt
2	PlateA.txt	PlateC.txt	

6. **If the input file is the Consolidated type, no additional files should be added.**
   All sample plate information should be within the first input file (that is currently in the Column 1, Line 1 cell). Remember, the data for the sample plates in this file must be in the same order in which the plates were used for printing!
7. **To delete a file from the Input File chart, select the cell with that file and click the Remove button.**

29.1.4 Output file

1. **Select a Destination File Type from the pull-down menu.**
   Currently GeneMachines offers five output file types. Three of these types are specifically formatted for scanner/ analysis programs. Prior to choosing an output file type, it is important to understand the slide orientation and notations that are used in the deconvoluted files. ***Please read the rest of this section, starting with 29.1.4.1 "Slide orientation" to learn about the notations and file types.***
2. **To select a name and location for the destination (output) file, click the BROWSE button.**
   In the browswer window open the location to which the file should be saved. Type in the name and click **OPEN**. (It is not necessary to type the file extension for the name.)

29.1.4.1 Slide orientation

For this sample tracking software, GeneMachines uses the slide orientation shown in Figure 29-5. If frosted slides were used, it is assumed that they were oriented such that the frosting was on the left side of the holder during printing. **The slide orientation is then rotated 90° counter-clockwise for deconvolution.** This means that the information in the deconvolution file will apply to the slide in this orientation.

29.1.4.2 Subarrays and Blocks

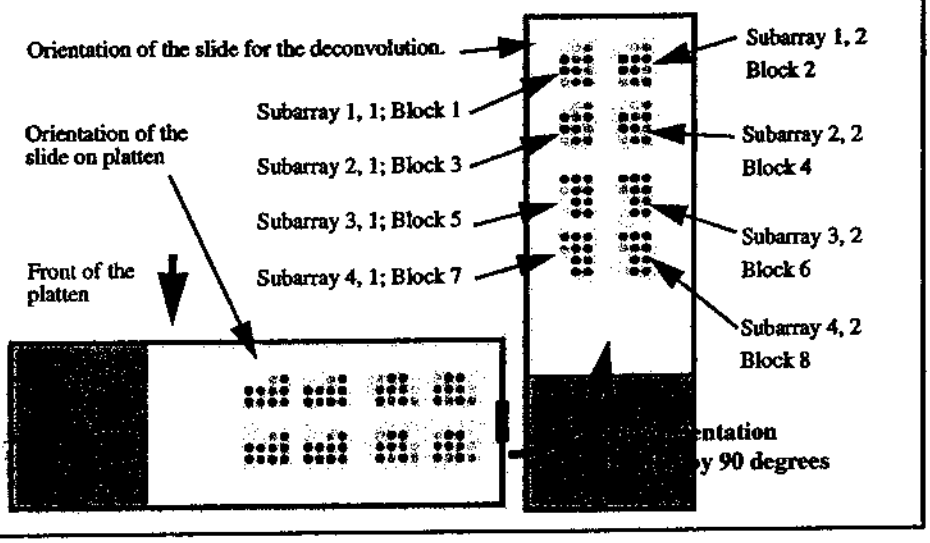
In some of the output files, the user will see the word *'subarray.'*

This term refers to the discrete set of spots printed by one pin. If the user has slide with two arrays that were printed with 16 pins, there should be 32 subarrays on the slide. They are identified by two coordinate values (see Figure 29-5).

In other output files, discrete sets of spots will be labeled as *'blocks.'* (These are *NOT* the same as the sample blocks described in the Build Methods section of the manual!)

Functionally, these blocks are the same as subarrays. (For example, in the example given above, there would be 32 blocks). The difference between the two notations is that blocks will only have one reference number (as opposed to the two coordinate values used for subarrays). Assuming that the slide has already been turned 90° counter-clockwise, the numbering starts at the top left block and progresses row by row (see Figure 29-5).

FIGURE 29-5 Slide orientation for deconvolution (sample tracking)



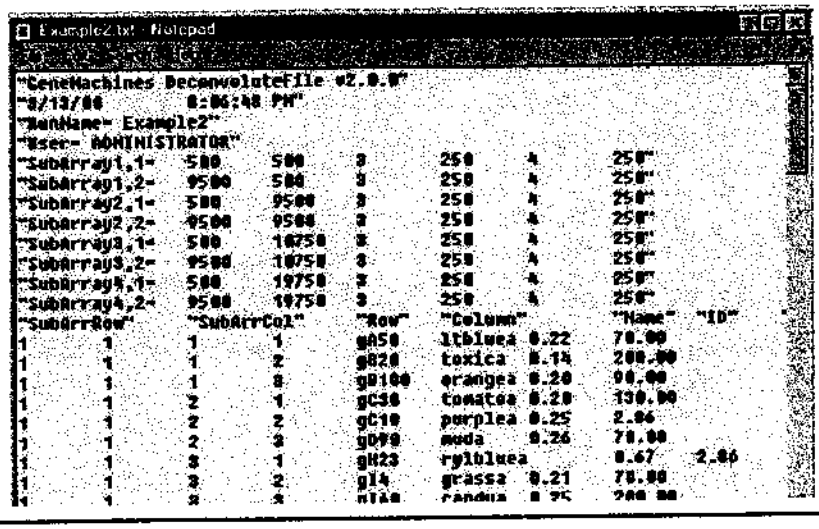
29.1.4.3 GeneMachines Type 1:

This output format is a tab delimited text file. This type passes the maximum number of fields from the input files into the output file.

The header lines on the output file will have the following information:

- **Date and time** of deconvolution.
- **Runname** (this is the name of the method selected in the Deconvolution screen).
- **User** (this will be the name that was entered when logging in to the software)
- **Subarray information** (The GeneMachines Type 1 output format refers to the discrete sets of spots as subarrays. The header lists each of the subarrays on the slide and provides information about the location and parameters of these files. The columns are as follows: subarray coordinates, X-direction location (on the slide) of first spot in the subarray, Y-direction location of the first spot,

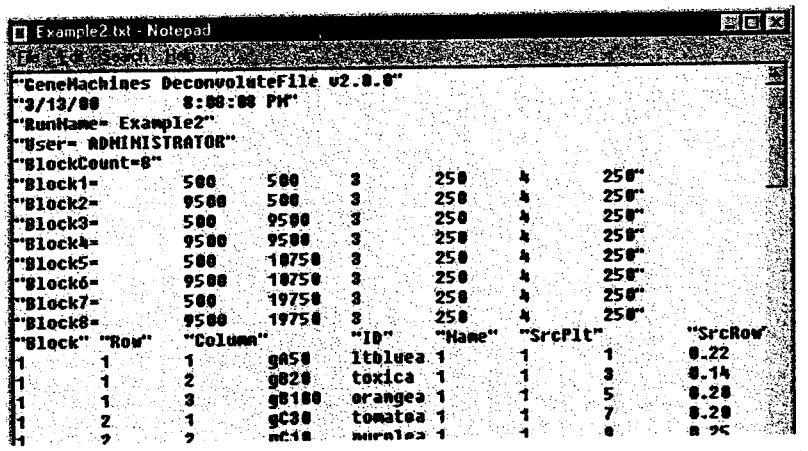
FIGURE 29-6 GeneMachines Type 1 output file



number of spots in the X-direction, dot spacing in the X-direction, number of spots in the Y-direction, dot spacing in the Y-direction.)

After the header information, the file will have columns of information to describe each spot in the array.

FIGURE 29-7 GeneMachines Type 2 output file



29.1.4.4 GeneMachines Type 2:

Like the other GeneMachines output format, this file type passes through the maximum number of fields into a tab delimited text output file.

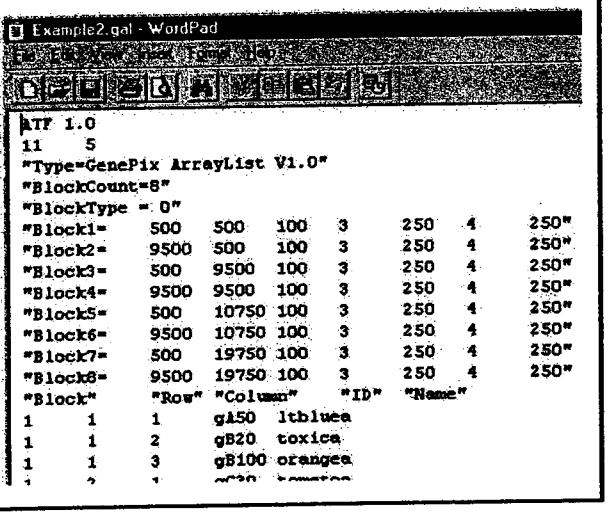
The two main differences are:

- **This format uses blocks rather than subarrays and**
- **There are three additional columns between Name and Opt1.**

(These three columns are SrcPlt, SrcRow and SrcCol which represent source Plate, Row and Column for the corresponding sample position in the source plate.)

Otherwise the header and spot information portions will have the same information as a Type 1 output file (see Figure 29-7).

FIGURE 29-8 Axon GenePix ArrayList output file



29.1.4.5 Axon® GenePix™ ArrayList:

This file format is directly compatible with Axon Instrument's scanner software. It is very similar to the GeneMachines Type 2 format with a few exceptions:

- **Unlike the other output files, this type will be a '.gal' file.** The output file will include a header that uses blocks to reference the discrete sets of spots.
- **The Axon format will not include the two columns of optional information (Opt1 or Opt2).**
- **The user should enter the Feature Diameter, or spot size, (in microns) for the printed array(s). (The feature size option will be disabled for the other output file types.)** The Axon software uses this information to create a grid that will be automatically superimposed over the array image when the user loads the deconvolution file. It will not be necessary to redo the deconvolution if the feature size is not accurate -- the user will be able to resize the grid in the Axon software!

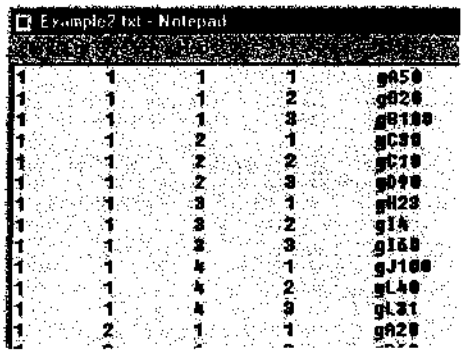
29.1.4.6 BioDiscovery® ImaGene™:

This output format allows the file to be imported into the BioDiscovery ImaGene software.

It has the following features:

- **This output format is a tab delimited text file.**
- **The file uses subarrays.**
- **It will not include a header or the Name or optional columns (Opt1 and Opt2) because these are not compatible with the BioDiscovery software.**

FIGURE 29-9 BioDiscovery, GSI



29.1.4.7 GSI Lumonics®:

This output format allows the file to be imported into the GSI Lumonics QuantArray® software.

This format is the same as the BioDiscovery file type.

29.2 SAMPLE TRACKING: PLATE LOADER VERSION

1. **Click on the SAMPLE TRACKING button in the Select Gridder Operation menu.**
   This will open the Sample Tracking menu for the plate loader version (see Figure 29-10).

   The major difference between this version of the Sample Tracking menu and that of the non-plate loader is in the Input File chart.

   The formats for input and output files remains the same as described above. This following information will reference any overlapping information from previous sections.

29.2.1 Input parameters

1. **From the first pull-down menu, choose the Plate Sequence name that was used for the printing run.**
   Next to the plate sequence name, the software will display the total number of plates in that chosen sequence.
2. **Next select the Run Method that was used from the second pull-down menu.**
   The software will display the total number of plates saved for that method next to the name.

> ***Note:*** **Remember that if the plate sequence or run method has been changed since the samples were printed onto the slides, the deconvolution may not be done correctly!**

3. **After selecting these two options, the software will indicate how many plates positions are available for the deconvolution.**
   If the number of plates in the selected plate sequence do not match that of the run method, the software will use the lesser number.
4. **Please refer to Section 29.1.1 for a description of the Feature Diameter option.**