



Version 7.0

# Application Guide: Gel Analysis

## **PROPRIETARY NOTICE**

This MCID™ Basic software and all associated documentation ("MCID Basic") constitute valuable assets of, and are proprietary to, Imaging Research Inc. ("Imaging Research") and are protected as such under both the law of trade secret and copyright.

No part of MCID Basic may be made available or disclosed to anyone without the prior written consent of Imaging Research.

The use of MCID Basic and the liability of Imaging Research, if any, arising out of the use of this program by you is governed by the applicable software license agreement.

**Imaging**  
Research Inc.



# Contents

<b>Introduction</b> .....	<b>1</b>
<b>Density Profile: Basics</b> .....	<b>2</b>
Acquiring a Density Profile .....	3
Performing a Baseline Subtraction .....	6
Quantitation of Peaks/Bands .....	7
Profile Measures .....	10
Managing Data .....	11
<b>Density Profiles: Advanced Details</b> .....	<b>12</b>
Graph Control Icons .....	12
Auto Lane Tool Options .....	20
Labeling Data .....	21
Data Display Options .....	22
Other Profile Measures .....	23
<b>Direct Band Analysis: Basics</b> .....	<b>23</b>
Sample Tools .....	24
Establishing a Background Density Value .....	25
Gathering Data .....	25
Labeling Data .....	26
Reference Data .....	27
<b>Direct Band Analysis: Advanced Details</b> .....	<b>28</b>
Creating Your Own Lane or Band Labels .....	28
Data Display Options .....	28
Other Measures .....	29
<b>Calibrations</b> .....	<b>29</b>
Uncalibrated and Calibrated Images .....	29
Density Calibration: The Step Tablet .....	30
Spatial Calibrations .....	31
<b>Creating Custom Data Reports</b> .....	<b>33</b>



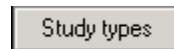
## Introduction

The **Gels: Lane Analysis** study type is used for the analysis of one-dimensional gels and blots (e.g., Northern, Southern, Western). To enable the study type:

1. Select *Settings > Study > Gels: Lane Analysis*.

**OR**

1. Click the **Study types** button at the bottom of the Shortcut Bar:



2. Click the **Gels: Lane Analysis** shortcut icon:



**MCID™ Basic** provides two different data gathering modes for this type of analysis:

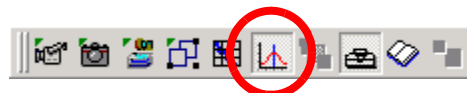
- Density profile
- Direct band analysis

In “Density profile” mode, **MCID Basic** generates a graphical display of density values (i.e., a density profile) across or within lanes. Numerical data are then obtained by sampling the density profile. Profile measures include density, position, area under curve, % highest density value, and more. Features are also provided for “smiling” corrections and for automatic peak detection.

In “Direct band analysis” mode, **MCID Basic** is configured to gather data directly from the image without obtaining a density profile. Direct measures include density, area, position, and integrated area (i.e., density x area).

Both data gathering modes offer a form of automatic background/baseline correction and data can be expressed relative to a reference band. Calibration to nonlinear Position standards (e.g., molecular weight) is also possible in either mode.

When you select the **Gels: Lane Analysis** study type, **MCID Basic** is configured by default to generate a density profile. If you want to perform direct band analysis, de-select the **Profile** icon located in the **Application** toolbar



**MCID Basic** loads the appropriate measures for direct band analysis. To return to profile mode, click on the **Profile** icon again.

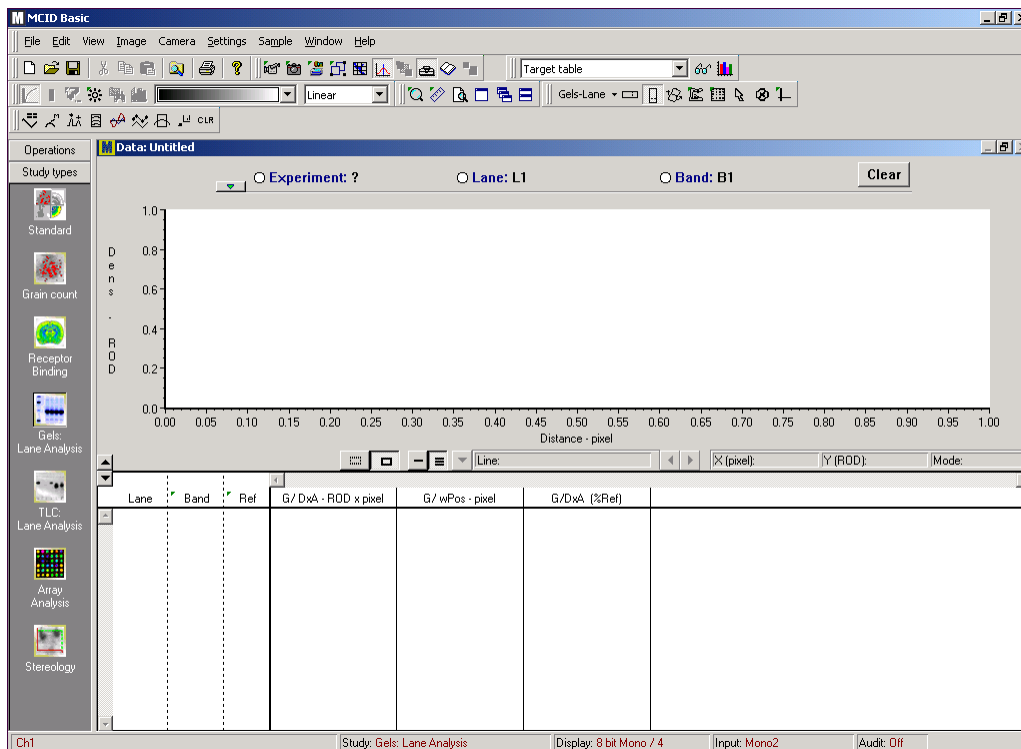
Although Direct band analysis is more straight-forward than Density profile, it also uses a much simpler background correction process. Use direct analysis only when the signal from the bands of interest is high (relative to the background of the gel) or when accuracy is not critical.

## Density Profile: Basics

When you select the **Gels: Lane Analysis** study type, the **Sample** window is automatically configured for profile analysis (Figure 1). The window consists of:

- a graph area where image density values are plotted as a function of distance or position
- a set of controls for displaying single or multiple profiles
- a data table where numerical profile data will be reported
- a status bar

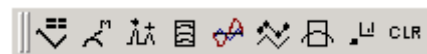
**Figure 1:** In Profile mode, the *Gels: Lane Analysis Study* type configures one area for displaying graphs and another area for displaying numerical data.



Two specialized sets of tools also appear. The Image Tools toolbar (*View > Image Tools Bar*) now contains a set of **Gels-Lane** tools for creating density profiles:



The Table toolbar (*View > Table Bar*) contains a set of “graph control” tools that control the display of the density profiles:



Either of these toolbars can be dragged into the **Sample** window for easier access (as in Figure 2).

The analysis of electrophoresis gels and autoradiograms involves the following general steps:

- Digitize the specimen.
- Make all necessary calibrations.
- Acquire a lane density profile.
- Perform a baseline correction.
- Define and quantify peaks (bands) on the profile.

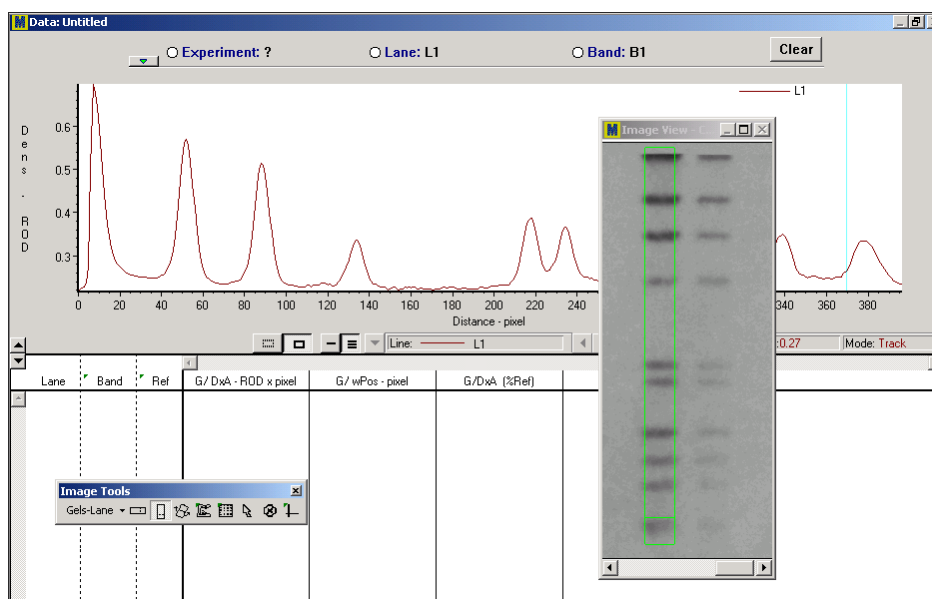
You can use manual or automatic peak definition and quantification. Data are reported to the data table.

### Acquiring a Density Profile

To acquire a density profile, first select one of the **Lane** tools from the **Gels-Lane** toolbar (see below). Adjust the size of the **Lane** tool on the image for the region of interest. The region of interest may be a single lane, a particular band that appears in multiple lanes, or an entire gel (if you are using the **Auto Lane** tool). Position the tool over the region of interest and click the left mouse button. **MCID Basic** scans the length of the profile tool and plots density values as a function of distance or position. The profile appears in the graph area of the **Sample** window.

The **MCID Basic** imaging system's default uncalibrated density measurement unit is "relative optical density" (ROD). If you are working with UV gels (i.e., light bands on a dark background) change the density unit to "gray levels" before you acquire a profile, or peaks will be inverted. See the [Calibrations](#) section of this document for instructions. Instructions are also provided for calibrating ROD to an optical density step tablet.

**Figure 2:** Density profile of a single lane. The profile was generated from the GEL2.IM image file contained in the Standard Tutorial Data.



### Lane Tools

MCID Basic provides several types of lane tools in the **Gels-Lane** toolbar. Select the most appropriate tool for your specimen:



**Horizontal Box** – This tool scans density values from left to right. Use this tool when the lane is oriented horizontally. Define the width and length of the box by moving the mouse while holding down the <Ctrl> key.



**Vertical Box** – This tool scans density values from top to bottom. Use this tool when the lane is oriented vertically. Adjust the length and width of the box as described above.



**Rotatable Box** – This tool scans density values along a user-defined axis. To rotate it, move the mouse while holding down the <Alt> key. To alter the size of the tool, move the mouse while pressing the <Ctrl> key.



**Click-ribbon** – This tool scans along user-defined points. Adjust the width of the ribbon (circle) to the width of the lane by holding down the <Ctrl> key while you move the mouse. Click on the beginning of the lane and then on any points along its length. The width of the tool can be re-adjusted at any point.



**Auto lane** – This tool detects and scans lanes automatically. Adjust the size of the box by holding down the <Ctrl> key while moving the mouse. Place the box over the region of interest and click. MCID Basic automatically finds and scans all lanes that are contained in the region of interest. See the [Density Profile: Advanced Details](#) section for more information.

Although the following tools are also contained in the **Gels - Lanes** toolbar, they are not used to create density profiles.



**Pointer** – This tool is used for gathering data from a profile outline on the image. See the [Quantitation of Band/Peaks](#) section for details.



**Delete** – This tool deletes a density profile. To delete a profile, place the cursor inside its profile outline (on the image) and click.



**Pixel Info** – This tool is a cross hair with a single pixel in the middle. Position the cross hair anywhere on the image and click the left mouse button. The XY location and the density value for the pixel under the cross hair will be displayed at the top of the image. Use it to have a quick look at densities without actually taking data samples.

### *Positioning a Lane Tool Within a Large Image*

If you are working with a large image file (e.g., from a phosphor imager), you may not be able to fit an entire lane within the Image View. If this is the case, change the display magnification so that a larger portion of the image is displayed in the Image View.

1. Click the top left corner of the Image View to open the view window menu.
2. Select the **Magnification** option.
3. Set the magnification factor to **50%** or less.

A compressed version of the image appears in the Image View. Please note that although the image is compressed, the density profile is still obtained at full resolution.

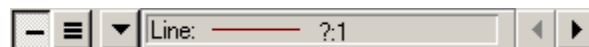
### Acquiring Multiple Profiles

A new profile appears in the graph area each time you click the **Lane** tool. To avoid confusion, different profiles are displayed in different colors and/or line types. By default, the **MCID Basic** labeling scheme is active, and a unique “lane” label (L1, L2, L3, etc.) is automatically assigned to each profile as it is acquired. If you wish to use another labeling scheme, see the “Labeling Data” section in [Density Profile: Advanced Details](#).

If you use the **Auto lane** tool, **MCID Basic** automatically generates a profile for each lane contained in the region of interest.

### Displaying Multiple Profiles

**MCID Basic** displays one profile at a time by default. Controls for displaying single or multiple profiles are located beneath the graph area:




Use the controls for moving between individual profiles or for displaying all profiles at once.

- Single** – Display profile associated with current lane label. All other “non-working” profiles are displayed as faint lines in the background.
- All** – Displays all acquired profiles.
- Select** – Selects a specific profile. A drop-down list displays the line type and lane label for all acquired profiles.
- Next** – Displays profile associated with next lane label.
- Previous** – Displays profile associated with previous lane label.

The two push-button icons to the left of the line display controls alter the display of the profile outline(s) on the image (i.e., the region from which the profile was acquired). Profile outlines must be turned on in order to obtain specific profile measures (e.g., G/DxA).

- On** – Displays one or more profile outlines on image.

 **Off** – Does not display profile outlines on the image.

If **MCID Basic** is configured to display one profile at a time, the active profile outline is displayed in red. Profile outlines for “non-working” profiles are displayed in green.

### Performing a Baseline Subtraction

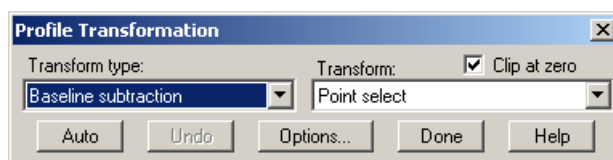
In a density profile, individual bands are seen as sharp peaks riding upon a slowly varying background or baseline. To obtain accurate density measures for specific peaks, you must exclude these irrelevant baseline density components (Figure 6).

Baseline subtraction is available by clicking the **Transform** icon in the graph control toolbar:



This displays the *Profile Transformation* dialog box (Figure 3).

**Figure 3:** The *Profile Transformation* dialog box is used to transform or manipulate a density profile. “Baseline subtraction” is selected here as the “transform type”.

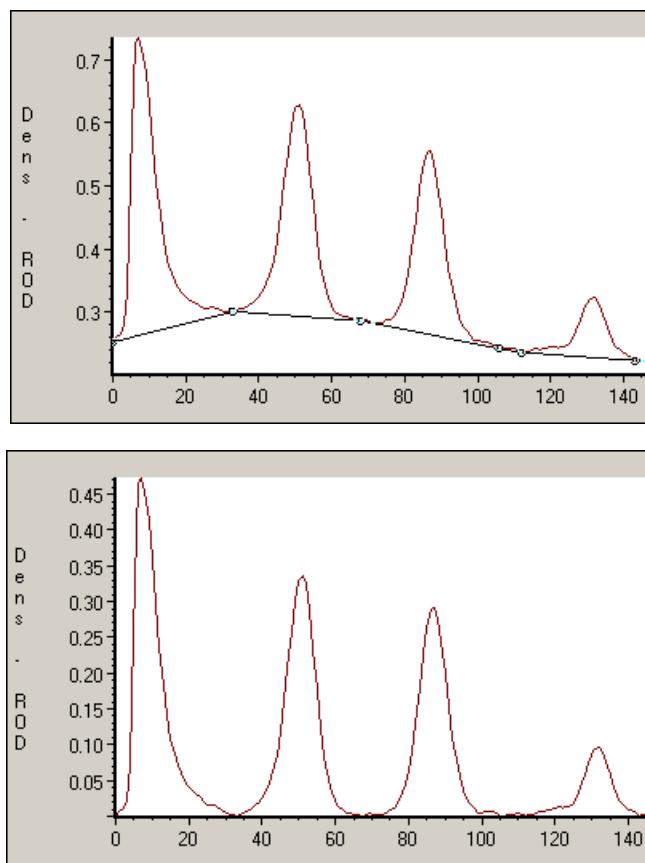


The easiest procedure is to click on [**Auto**], (when you do this, the [**Auto**] button becomes the [**Apply**] button). **MCID Basic** defines a reasonable baseline for the displayed profile(s), showing it to you as a line at the base of the profile graph(s). If you agree with the definition, click on [**Apply**] for the subtraction to occur. Click on [**Done**] to finish with the baseline subtraction or click on [**Reset**] to try other options.

You can edit the baseline at any time before clicking [**Apply**]. You can add additional points by pointing to the profile and clicking the left mouse button or delete points by clicking on them with the right mouse button.

A complete description of baseline subtraction features and options is provided in the [Density Profile: Advanced Details](#) section.

**Figure 4:** Profile lines across a SDS PAGE lane, showing densities before (top) and after (bottom) baseline subtraction. In the top graph, much of the area comes from nonspecific density common to many peaks (baseline). In the bottom graph, the baseline has been removed and area measurements would more accurately reflect each discrete peak.



### Quantitation of Peaks/Bands

Quantitation is achieved by sampling the density profile. You can define peaks or bands and sample them manually or with the **MCID Basic** automatic peak detection feature. You can gather data from individual line points (e.g., the highest peak value) or from a section of the profile (e.g., an entire band/peak). Data (density, position, area under curve, and other measures) are reported in the data table beneath the graph area.

**MCID Basic** automatically assigns a “band” label to the data (B1, B2, B3, etc.) each time you sample a density profile. If you want to use another data labeling scheme, see the “Labeling Data” section in [Density Profile: Advanced Details](#).

If you are working with multiple profiles, you can gather data from one profile at a time or from all profiles at once. To sample all profiles simultaneously, click on the multiple line display icon before you sample:



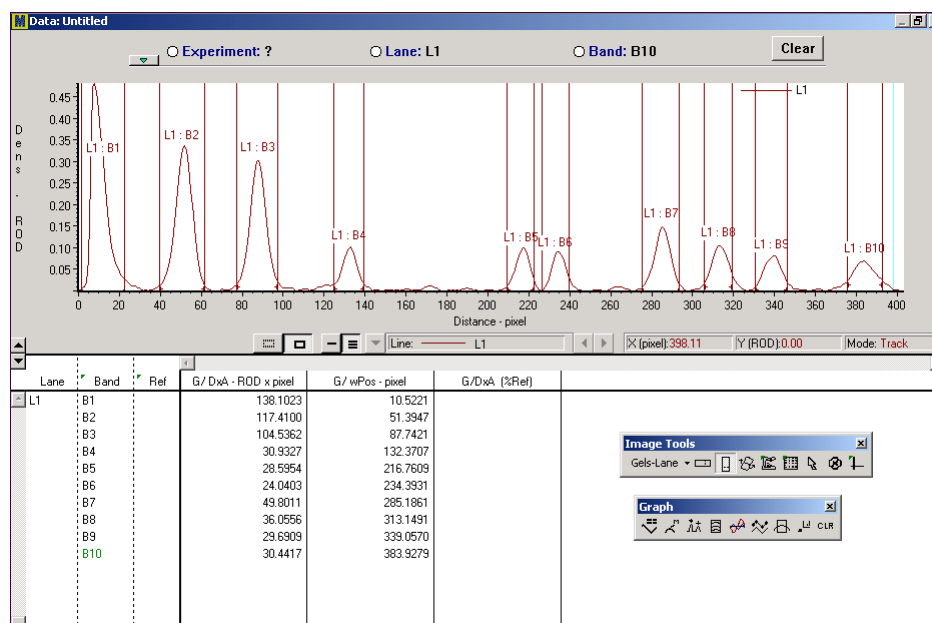
MCID Basic automatically sorts data from different profiles by their corresponding “lane” labels.

### Defining Peaks Manually

To sample a density profile manually, move the cursor into the graph area. A vertical blue line appears on the graph. This “line cursor” moves along the X axis of the graph as you move the mouse. Just under and to the right of the profile graph is a status bar showing the current cursor position and density value at that point. Notice that the numerical values in the status bar are continuously updated as you move the line cursor across the profile. Another moving line appears in the profile outline, and this also corresponds to the position of the line cursor on the density profile.

Use the line cursor to define points or peaks on the profile. To sample a single line point, position the line cursor over the point and click the left mouse button. Data from that point appears in the data table. To sample an entire peak, move the line cursor to one end of the peak, hold down the left mouse button and drag it to the other end. When you release the mouse button, peak data appear in the data table.

*Figure 5: An analyzed profile with a set of measurements.*



### Defining a Peak on the Image

You can also use the image as a reference for peak/band definition instead of using the density profile. To define a peak on the image, select the **Pointer** tool from the **Gels-Lanes** toolset:

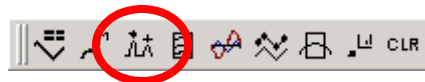


Place this cursor inside a profile outline in the image. If there is more than one profile outline, **MCID Basic** automatically displays the profile that corresponds to the selected profile outline.

The cursor is now attached to a line inside the profile outline. The vertical blue “line cursor” on the graph display marks the position of this line inside the lane. Move the line to one end of a band, hold down the left mouse button and drag it to the other end. When you release the mouse button, band data appear in the table.

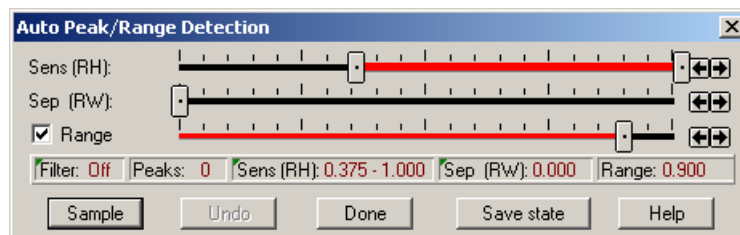
### Automatic Peak Detection

Automatic peak detection is an interactive function that detects peaks on a density profile, samples them, and reports the results to the data table. To use automatic peak detection, click on the **Peak Detection** icon in the graph control toolbar:



Clicking on the Peak Detection icon displays the *Auto Peak/Range Detection* dialog box (Figure 6). The *Auto Peak/Range Detection* dialog box contains controls for defining peak detection criteria. When **MCID Basic** detects a peak that falls within the acceptable range, it marks a small vertical line and a peak number through the highest point of the peak. When you are satisfied with the peak definition criteria, click on the **[Sample]** button to have the peak data scanned out and placed in the data table.

**Figure 6:** The *Auto Peak/Range Detection* dialog box is used to find and analyze peaks within profiles.



You can select peaks on the basis of **Sensitivity** (Relative Height) and/or **Separation** (Relative Width).

### *Sensitivity*

The **Sensitivity** criterion refers to the height of the peaks within a profile. Moving the left slider towards the middle decreases the sensitivity to small peaks (i.e., excludes small, irrelevant peaks from analysis). Moving the right slider towards the middle excludes larger peaks. The greater the distance between the slider controls, the wider the selection criteria will be.

### *Separation*

The **Separation** criterion is used to select peaks on the basis of their proximity to other peaks. Moving the slider towards the right excludes peaks that are close to each other.

The distance between peaks on the profile is affected by nonlinear distance calibrations (i.e., molecular weight). If your image is calibrated to a set of molecular weight standards, some peaks on the profile will appear closer together than they would had a linear distance scale been used. **Separation** criteria, however, do not use calibrated distance values to determine a peak's proximity to other peaks. Selection is based instead upon the number of pixels between peaks.

### *Range*

**Range** refers to peak boundaries, which are displayed as vertical magenta lines in the profile graph. If **Range** is not enabled, each peak is treated as a single point lying at the maximum value of the peak. This is useful for identifying peaks, but not for measuring peak areas.

To measure peak areas, boundaries for each peak must be established. **MCID Basic** does this automatically if **Range** is enabled. Moving the slider toward the left reduces the width of the peaks.

### *Editing the Automatic Peak Detection*

We have tried to make automatic peak detection as accurate as possible by allowing interactive definition of the peaks. Occasionally, you may need to edit the definition criteria for an individual peak without affecting the criteria used to define other peaks in the profile. **MCID Basic** provides a set of editing functions that allow you to add, delete, or shift individual peak definitions and adjust the defined range of individual peaks.

To add a new peak, position the cursor on the profile and press the left mouse button. A new peak marker appears. To delete a peak, position the cursor on a detected peak marker until the cursor changes to a horizontal double arrow and press the right mouse button. To move the peak marker to a new position, place the cursor on the peak marker until the cursor changes to a horizontal double arrow. Hold down the left mouse button and drag the peak marker to a new position.

To adjust the range, position the cursor on a peak boundary on the profile. When the cursor changes to a double arrow, hold down the left mouse button and drag the peak boundary to a new location.

Other automatic peak detection features and options are discussed in the [Density Profile: Advanced Details](#) section.

## **Profile Measures**

The following measures are reported as part of the default gel analysis configuration. In the data table, the word "Graph" is abbreviated as "G/".

**G/ wPos** – Weighted Graph Position. This is the position of the sampled line point, taken from the beginning of the profile. If an entire peak/band is sampled, the position is weighted toward higher density values. Position is calibrated to the current spatial unit (e.g., mm, molecular weight). If the image is not calibrated, position is expressed in pixels.

**G/ Dx<sub>A</sub>** – Mean density value of the sample line points, multiplied by the area of the corresponding sampling window on the image. This measure can be used as an estimate of signal intensity.

**G/ DxA (%Ref)** – G/ DxA, expressed as a percentage of the G/ DxA value of a reference band in the same lane. To designate a band as a reference, double-click in the reference (**Ref**) column beside that band's data. The reference column is the narrow column between the labels at the left and the numerical data at the right. To de-activate a reference, double-click in it again. One band per lane may be used as a reference.

In some situations it may be useful to designate the same band in every lane as the reference. This can be done simply by first reversing the band and lane columns in the data table before assigning the reference. To reverse the columns, simply double-click on the band column heading (where the green tab is). The band column will be displayed as the leftmost column. Now double clicking in the reference column next to the desired band (e.g. B2) will assign this band as the reference in all lanes.

See the [Density Profile: Advanced Details](#) section for a description of other useful profile measures.

## Managing Data

There are several different ways to manage the data on your screen. From the **Sample** window, you can:

- Print the data.
- Save the data to disk as a “log” file.
- Export the data as a text or spreadsheet file.
- Create a custom “data report”.

To print the data, simply open the *File* menu and select the *Print* option. Other data management options are discussed below.

## Generating Log Files

**MCID Basic** has an extensive set of data saving, editing, and management functions. To use these functions, you should save your data in “log files”. Select *File > Save as* while the **Sample** operation window is open. Give the file a unique name. **MCID Basic** automatically attaches an “.lg2” extension to the log file name and writes it to disk.

You can retrieve, edit, add to, and summarize data in log files at any time. The log file contains profiles, numerical data, and the calibration that was in effect when you last saved the log file.

## Generating Custom Data Reports

“Data reports” are a convenient way to summarize the results of an analysis session. With a single command, you can save or print images, density profiles, and numerical data from the profiles as a unified report file.

**Note:** Data reports do not replace log files. In contrast to a log file, a data report does not contain any calibrations and it cannot be edited at a later date. We recommend that you save your results in log files and use the data report function to summarize data for placing into notebooks.

Instructions for generating custom data reports are presented in the [Creating Custom Data Reports](#) section of this document.

### Exporting Data

You can save profile data as a text file, a Lotus® 1-2-3 \*.wks file or as a Microsoft® Excel \*.xls file. The exported text file may be treated as any text file (you can print it, import it into other programs, etc.). A variety of database and spreadsheet programs can accept Lotus or Excel file formats.

To export data from the **Sample** window, use the *File > Export* command. When the *Export Data* dialog box appears, select the desired file format (tab delimited text, comma delimited text, space delimited text, Lotus or Excel) and enter a filename. **MCID Basic** automatically attaches the appropriate extension (“.txt”, “.csv”, “.prn”, “.wks” or “.xls”) to the filename as it is saved to disk.

Because the text, Lotus or Excel files contain the data just as it appears on your monitor, use the data display controls to create the appropriate type of display before you export it. Data display options are discussed in the [Advanced Details](#) section.

## Density Profiles: Advanced Details

### Graph Control Icons

#### Clear



Deletes the “working” profile(s) from the graph area. If **MCID Basic** is configured to display a single profile, options are presented for clearing “non-working” profiles as well. This button does not delete data that you have gathered from the profiles.

#### Paste



Paste places the graph into the **Report** window, where you can manipulate it in various ways:

- Re-sizing by dragging the borders of the **Report** window.
- Copying to the clipboard, using *Edit > Copy*.
- Printing to the printer, using *File > Print*.
- Saving as a TIFF file, using *File > Export*.
- Exporting its numerical data (i.e., each data point on the line) in either text, Lotus or Excel format using *File > Export*.
- Cutting out the profile and placing it in the image using the **Cut** tool in the **Graphics** toolbox.

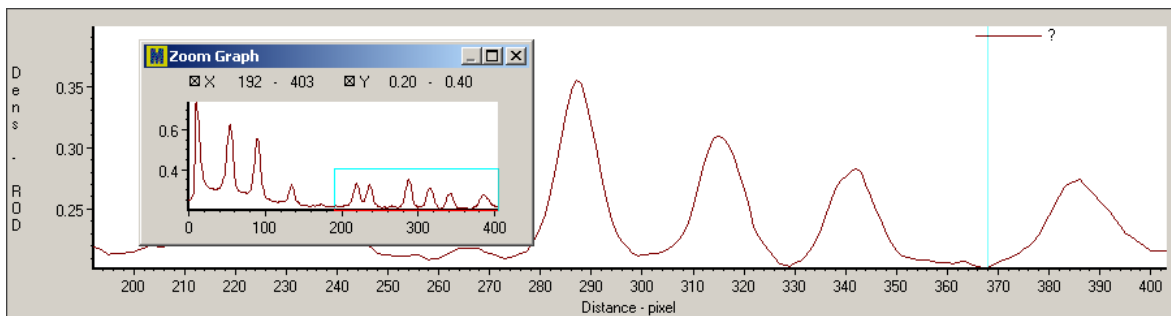
#### Range



The range control affects the display limits of the X and Y axes. By default, the axes automatically display the full range of data within the profile. Using the range control, you can set the X and Y limits to display any portion of that range.

Suppose, for example, that you obtain data whose full range on Y is 0.0 - 0.9 ROD. It may be difficult to see small density differences on the graph if you display the entire range. To help see small density variations, you can set the display range to show a small part of the range in much more detail.

**Figure 7:** A profile display with a range control window. The top border of the range window has been dragged down toward the baseline. This creates a small box that defines the main graph area that is being displayed at high detail.



### Setting the Axes

There are two ways to set the X and Y axes:

1. Click on the numbers at the top of the range control window and type values to define new limits for one or both of the axes.
2. Drag the borders of the range control window. When you place the cursor on any edge of the box the cursor becomes a double arrow. Use this to drag the edge of the range control and change the axes.

Once a range is defined by either of the above methods, you can move the entire range around by placing the cursor inside it and dragging.

### Profile Options



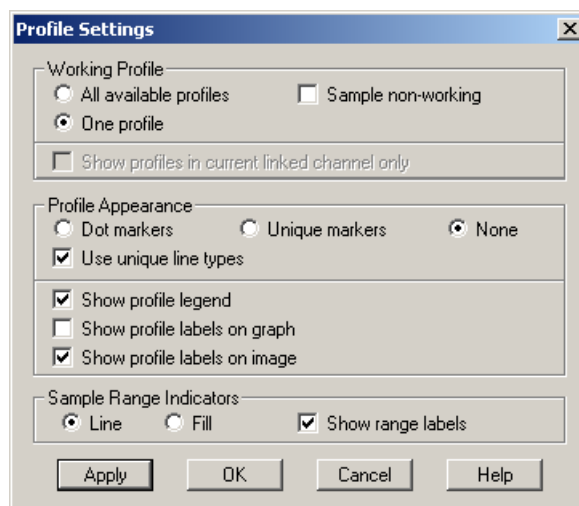
Controls the profile display, appearance, and labeling by using the *Profile Settings* dialog box.

### Working Profile Options

Controls for displaying single or multiple profiles are located beneath the graph area. Additional controls are available in the Line Settings dialog box. When the **All available profiles** option is selected, **MCID Basic** displays all profiles acquired under all lane labels. When **One profile** is selected, **MCID Basic** displays only the profile(s) associated with the current lane label. Profiles from other lane labels (i.e., “non-working” profiles) are displayed as faint lines in the background.

When the **Sample non-working** option is selected, **MCID Basic** will gather data from all profiles simultaneously, even if it is configured to display a single profile.

*Figure 8: The Profile Settings dialog box gives access to options regarding profile display.*



### *Markers, Labels, and Legends*

By default, different profiles are displayed in different colors and line types (e.g., a solid red line, a blue dashed line, etc.). Although individual data points are not marked by default, markers are available. Markers are characters (e.g., dots or crosses) that mark the position of each data point on the line. Select **Unique markers** to have each profile use a different type of marker. Select **None** or **Dot markers** for a less cluttered display.

Select **Show profile legend** to display the lane label associated with profile line type. The legend is displayed at the top, middle, or bottom of the graph, either in a horizontal line or arranged vertically in columns. See below for details.

Unlike legends, profile labels appear within the body of the graph with an arrow pointing to their corresponding profile. When you select **Show profile labels on graph**, **MCID Basic** labels each profile at its left end (with its corresponding lane label). If you select **Show profile labels on image**, **MCID Basic** labels the profile outlines on the image.

### *Sample Range Indicators*

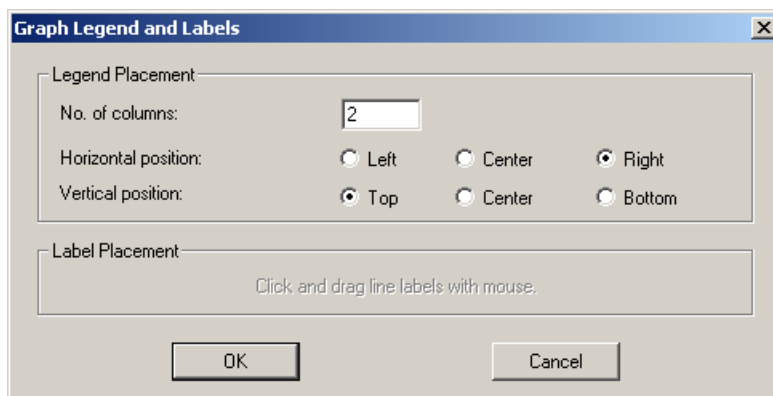
When you gather data from a profile, **MCID Basic** marks the beginning and the end of the sampled region (i.e., the sample range). The sample range can either be marked with vertical red **Lines** or completely **Filled** with red. When you select **Show range labels**, **MCID Basic** displays the lane and band labels that correspond to each sample range.

### **Graph Legend**



You can display the profile legend at the top, middle, or bottom of the graph, either in a horizontal line or arranged vertically in columns.

**Figure 9:** The Graph Legend and Labels dialog box controls the placement of the graph legend.



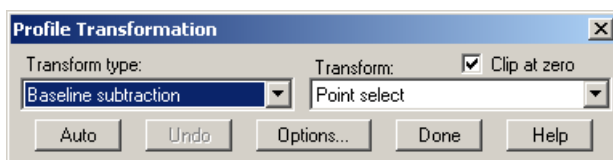
## Transform



You can manipulate a defined profile in many ways. Some of the manipulations simply change the appearance of the graph. For example, changing the range allows you to look more closely at a limited portion of the graph. Other manipulations change the data values in the graph. These manipulations are termed “transforms”.

MCID Basic has three types of transforms: **Baseline subtraction**, **Translation**, and **Filters**. Use the *Profile Transform* dialog box to select different transforms and their options.

**Figure 10:** Use the Profile Transform dialog box to select different transform functions (Baseline subtraction, Translation, Filters) and their options.



### **Baseline Subtraction**

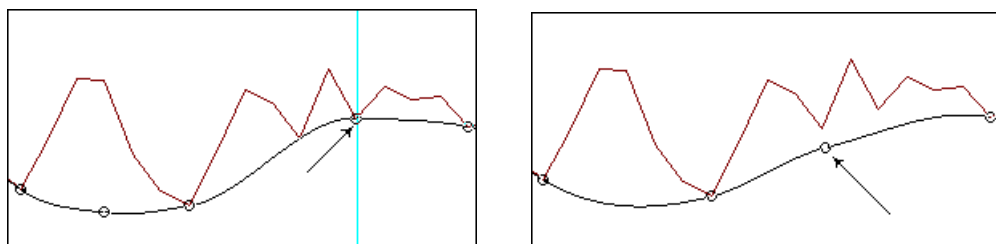
Baseline subtraction and automatic baseline setting were discussed in the “Basics” section. We discuss more flexible aspects of baseline subtraction in this section.

You can place baseline points manually or automatically. Manual tracing allows you to place baseline definition points at any XY locations on the profile. Starting on one side of the graph, click on a point that corresponds to baseline. Move the mouse to the next baseline point and click again. You may pick any position in X (distance) and Y (density). A line appears connecting each baseline point to previous points. This line defines what you consider to be the true baseline.

To add a baseline point, click the left mouse button. To remove a point, move the cursor over that point and click the right mouse button. Continue until you have defined enough points to create an accurate baseline. Move the mouse out of the graph area. Click on **[Apply]** to complete the subtraction.

The *Transform* drop down menu contains two methods for manual baseline definition: **Point select** and **Point define**. Use point select to have baseline points appear on the profile. Choose point define to have baseline points appear wherever you click.

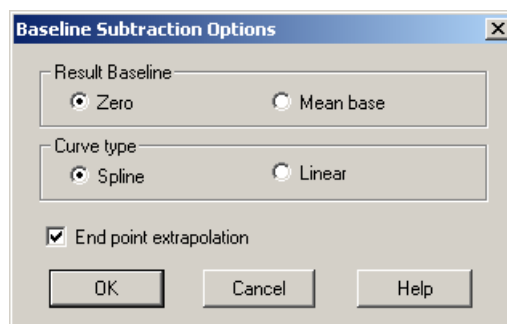
**Figure 11:** Using 'Point select' and 'Point define' for automatic baseline definition and editing. The baseline in both graphs was defined automatically. In the left panel, an additional baseline point (arrow) was added using point select. Notice that the point is placed onto the profile, above where it was clicked onto the graph. In the right panel, the additional baseline point remains where it was placed (point define).



### Baseline Subtraction Options

When you subtract baseline variation, you bring the entire profile graph down towards the X axis. There are various ways to do this. View your options by pressing the **[Options]** button. These options apply to both manual and automatic baseline definitions.

**Figure 12:** Options for baseline subtraction.



When you select **Zero** as the **Result Baseline**, **MCID Basic** removes baseline and shifts the entire profile down so that the minimum point is at zero ROD. If **Mean base** is selected, **MCID Basic** calculates a mean baseline value for the entire profile before subtraction. It performs the baseline subtraction and then shifts the profile line back up so that it centers on the original mean baseline value. This has the effect of removing regional variation from the profile line while maintaining densities at about the same values they were before baseline subtraction.

If **Linear** is selected as the **Curve type**, **MCID Basic** draws a straight line between consecutive baseline points. When **Spline** is selected, **MCID Basic** fits a cube root function through the baseline points.

Enable the **End point extrapolation** option to extend the baseline beyond the last baseline point on the profile.

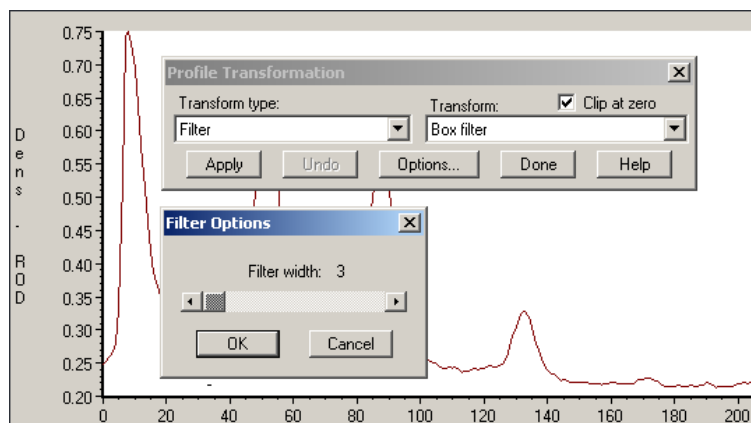
### Translation

Multiple profiles may be misaligned with respect to each other. To correct this, select **Translate along x**. Move the mouse cursor into the graph area and drag a profile from side to side. When it is aligned, click **[Apply]**. Select the next profile and repeat. Translations in the Y axis or in a combination of X and Y are also available.

### Filters

A filter is a transformation that produces a smoother or sharper density profile. **MCID Basic** provides **Box** and **Triangular** filters for smoothing a jagged line. Press **[Options]** and adjust **Filter width** to change the amount of smoothing. The wider the filter, the greater the smoothing effect. You are also free to enter any one-dimensional spatial filter by selecting **User defined** from the **Transform** drop down menu.

**Figure 13:** Profile line transforms have been used to select a Box filter. The **[Options]** button has been pushed to display the Filter Options dialog box, which shows a filter width of 3. Wider filters have a greater smoothing effect.



### Clip at zero

Any of the above transformations may cause some points on the profile to fall below the zero density value. Suppose, for example, that you translate a line in Y (shift it down towards the X axis). Some parts of the line may now lie below the X axis. If you clip these values at zero, you will truncate any parts of the graph that lie below the zero density line. These parts of the graph simply vanish and would not be used in calculating the area of peaks. If **Clip at zero** is not selected, the negative density areas are retained in the graph. This could lead to problems with density measurement although peak areas would be accurately represented.

### Smiling Correction



Smiling correction scans a lane and determines curvature (smiling) for each band. Once you have a density profile, click on the **Smiling correction** icon. Keep the image in view and click on the **[Auto]** button when the *Smiling Correction* dialog box appears. **MCID Basic** then draws green 'correction lines' through the bands on the image.

You can modify, delete or add the correction lines to bands that did not have enough contrast to be detected automatically. To modify a correction line, click on it with the left mouse button. Three control points appear on the line. Use these points to drag the line to any desired position. To delete a line, click on it with the right mouse button. To add a correction line to a band, click on the band while holding down the <Ctrl> key.

When you are satisfied with all of the smiling corrections (i.e., when the correction lines are aligned with the crooked bands), click the **[Apply]** button. The density profile will then shift accordingly. Click **[Done]** to exit. When you read data from a smiling band, the sample cursor will be automatically adjusted to follow the curvature of the band.

## Peak Detection

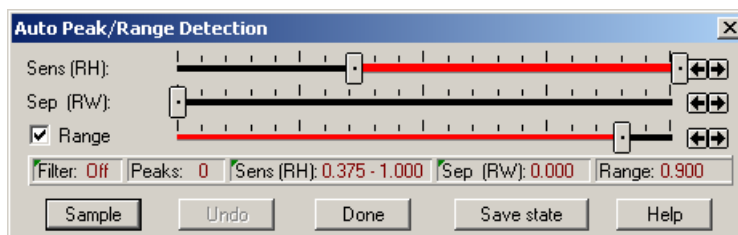


Automatic peak detection, quantitation, and settings of sensitivity and separation criteria were discussed in the “Basics” section. We discuss more flexible aspects of peak detection in this section.

### *Relative versus Local Detection Methods*

Sensitivity and separation criteria may be based on “relative” properties (i.e., Relative Height, Relative Width) or on “local” properties. The **Sep** and **Sens** fields in the status bar of the Auto Peak/Range Detection dialog box indicate whether local (**LH**, **LW**) or relative (**RH**, **RW**) attributes are being used. Double-click on either of these fields to change methods.

**Figure 14:** The “Sep” and “Sens” fields in the status bar of the Auto Peak/Range Detection dialog box indicate whether local or relative detection methods are in effect. Double-click on either of these fields to change methods.



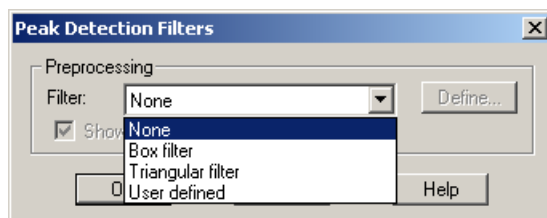
If the sensitivity criterion uses **Relative Height (RH)**, peak detection is based upon the peak’s absolute height relative to the maximum value on the profile. If **Local Height (LH)** is used, the detection criterion is based on a combination of Relative Height and the peak’s largest slope size. The local criterion eliminates small peaks that sit on a large baseline area sooner than the relative height criterion.

If the separation criterion uses **Relative Width (RW)**, peak detection is based on the distance between two adjacent peaks, relative to the width of the entire profile. If **Local Width (LW)** is used, detection is based on the valley height, largest slope, and distance between two adjacent peaks. This local criterion eliminates overlapping peaks sooner than the relative width criterion.

### **Filter: On or Off**

A filter is a transformation that takes a profile as its input and produces a smoothed or sharpened profile as its output. The **Filter** field in the status bar at the bottom of the dialog box) indicates whether or not the profile has been filtered. Double-click in the **Filter** field to display the *Peak Detection Filters* dialog box.

**Figure 15:** The *Peak Detection Filters* dialog box allows smoothing or sharpening of profiles prior to peak detection.



The **Box** and **Triangular** filters smooth profiles that are too jagged. Press the [**Define**] button to use or create a unique line filter.

Because the filter will be used during the peak detection process, select it before using peak detection. The result of the filter will be displayed with the original profile (if **Show filtered signal** is enabled).

### **Text Markers**

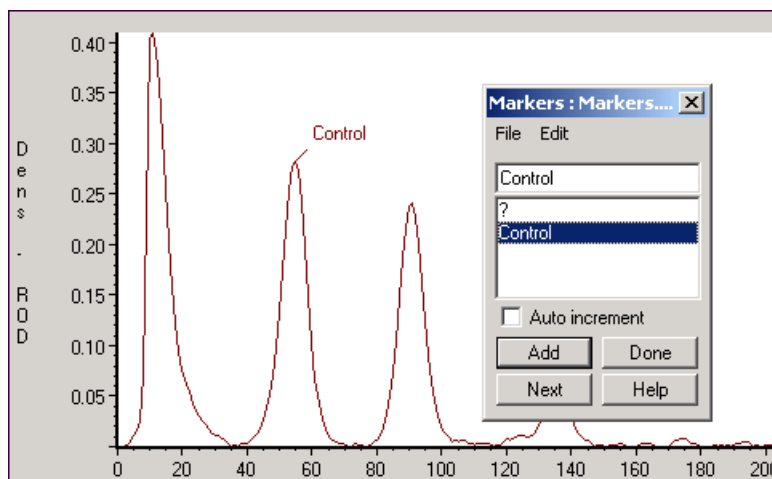


The **Text Marker** allows you to place specific labels or comments directly on the profile. Each marker, which can be placed anywhere on the profile, is limited to 31 characters.

To create a text marker, enter the text in the entry field of the Markers list box and click the [**Add**] button. The marker appears in the list below the entry field. You can enter as many markers as you wish. To place a marker on the profile, highlight the marker in the marker list and move the cursor into the graph area. Position the cursor over the desired point on the profile and click. The text appears above the profile with a small indicator pointing to the profile point. If the **Auto increment** option is checked, **MCID Basic** will place the next marker in the list wherever you click next. Press [**Done**] when you have finished placing the markers on the profile. The marker also appears on the profile outline on the image.

To edit or delete a marker, press the **Text Marker** icon to display the Marker list box. To edit a marker, highlight the marker in the marker list and open the *Edit* menu on the list box. Select *Change* to edit the text or select *Delete* to remove it. To re-position a marker, place the cursor in the graph area and position it over the marker. When the cursor turns into a double-arrow, drag it to a new position on the profile. Press [**Done**] when you have finished editing the markers.

**Figure 16:** A text marker on a density profile. A “Control” marker was entered into the Marker list box and then positioned on the second peak in the profile.

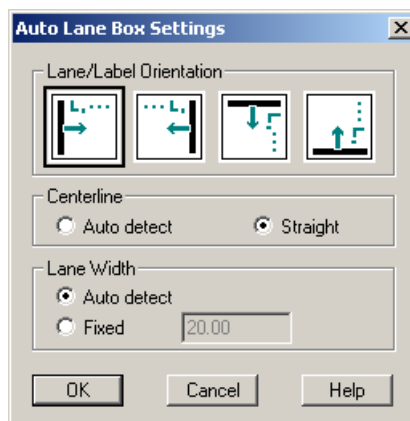


### Auto Lane Tool Options

The **Auto Lane** tool, which detects and scans lanes automatically, was described in the “Basics” section of this document. We discuss more flexible aspects of the **Auto Lane** tool in this section.

Ctrl-click on the **Auto Lane** tool icon to open the tool’s optional settings dialog box. The *Auto Lane Box Settings* dialog box appears.

**Figure 17:** Use the *Auto Lane Box Settings* dialog box to control the direction, orientation, and lane width of the automated scan.



The **Lane/Label Orientation** option controls the direction in which the lanes are scanned. Lanes may be scanned from left to right, right to left, top to bottom, or bottom to top. Match this setting to the way your gel is oriented. A cursor inside the **Auto Lane** box reflects the selected scanning direction. Lanes are labeled in consecutive order in the direction that they are scanned.

When the **Centerline** option is set to **Auto detect**, **MCID Basic** will follow any curvature that may exist in a lane. If set to **Straight**, **MCID Basic** will scan in a linear direction. You can rotate the **Auto Lane** box, however, to match your sample, if necessary.

If **Lane width** is set to **Auto detect**, **MCID Basic** automatically adjusts the width of the lane to match the average width of the bands contained in it. If you prefer to use a **Fixed** lane width, enter the dimensions in the entry field.

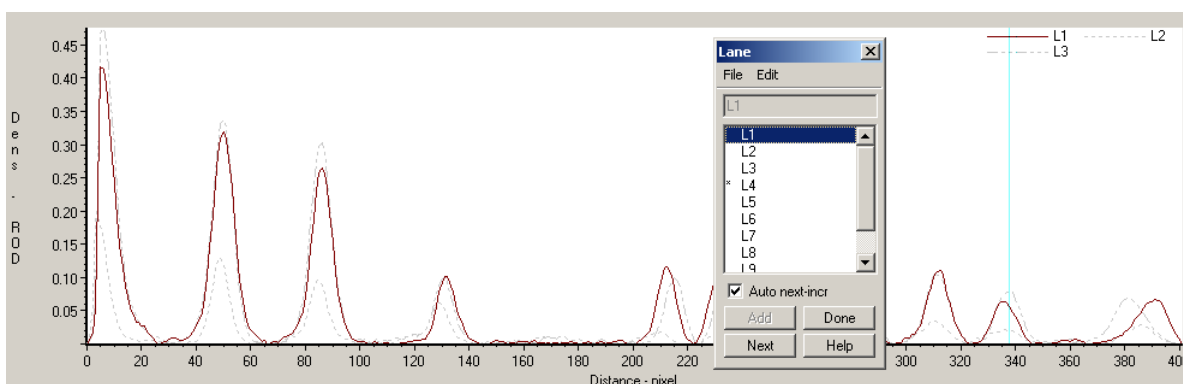
## Labeling Data

**MCID Basic** tracks gel data by **Experiment**, **Lane**, and **Band** labels, which are located at the top of the Sample window. You can use the default **MCID Basic** labeling scheme or create your own labels.

**Experiment** labels are used to designate individual gels or images. The default label is “?”. **Lane** labels are used to designate individual profiles. By default, **MCID Basic** assigns a lane label to each profile as it is acquired (L1, L2, L3, etc.). **Band** labels are used to designate regions of interest on the profile. By default, **MCID Basic** will automatically assign a band label to the region of interest as it is sampled (B1, B2, B3, etc.).

To view a list of default labels, click on the label heading at the top of the screen. A dialog box appears listing all of the labels for the selected label type. The current label is highlighted and an asterisk designates the next label that **MCID Basic** will use. The dialog box remains in place until you press **[Done]**. Initially the list contains only 10 labels, but as you sample and reach the last label in the list, another 10 are automatically added.

**Figure 18:** A Lane label dialog box. The highlighted label (L1) corresponds to the displayed profile. The asterisk designates the label that **MCID Basic** will assign to the next profile that is acquired (L4).



## Creating Your Own Labels

To clear the default **MCID Basic** labels and create your own, select *File > New* from the menu within the dialog box. The label list now contains a single “?” as a label. To enter a new label, place the cursor in the label entry field and type the new label name. This name becomes the current lane or band label and is added to the label list.

For example, suppose you wish to replace the default lane labels with labels that reflect specific cell lines (e.g., Att20, GH3, P19 and Cos-7):

1. Click on the “Lane” label at the top of the **Sample** window. The Lane label dialog box appears with the default lane label list.
2. Open the *File* menu on the Lane label dialog box and select *New*.
3. Enter the label “Att20”

4. Acquire a density profile from the Att20 lane.
5. Repeat steps 3 and 4 (using the appropriate lane labels) until all lanes have been scanned.
6. You do not have to retype a label once it is present in the list. Simply click on it to make it the currently active label.

The **Auto next increment** button directs **MCID Basic** to automatically step down one position on the Lane label list each time a profile is acquired. On the Band label list, the **Auto next increment** button directs **MCID Basic** to automatically step down one position on the Band label list each time a sample is taken from the density profile.

### *Saving and Editing Label Lists*

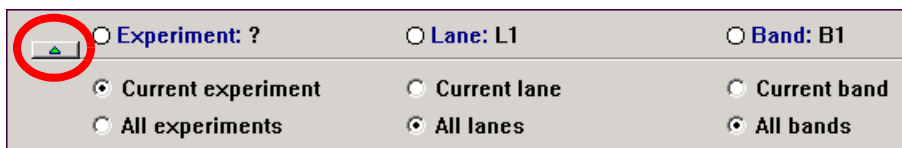
Label lists can be saved to disk, retrieved, and edited. The *File* menu in the label box allows the usual file manipulations of the label list (new/open/save/save as). Label list files are stored with an “LBL” extension. Because all label list files have the same extension, *do not save a Lane label list with the same name that you gave to a Band list.*

- To edit label lists use the *Edit* menu:
- To delete the selected label use *Delete*.
- To change the selected label use *Change*. For example, we might want to change an experiment label of “Gel 10” to “Gel ten”.

### **Data Display Options**

By default, the **Sample** window displays data gathered from all bands in all lanes from one experiment (i.e., all data obtained from one gel). This is the **Basic** data display for gel analysis. If you have gathered data under different Experiment labels, you must select the appropriate Experiment label in order to view its data. You can use the **Extended** data display to change the display configuration, however, so that data from all experiments are displayed in the data table.

Options for **Extended** data displays are located under the Experiment, Lane, and Band headings at the top of the screen. By default, these display options are hidden from view. To switch between the **Basic** and the **Extended** data displays, click on the arrow button to the left of the Experiment heading, as indicated below.



The **Extended** data display controls allow you to select one or more experiments, lanes, and bands. If you want to display data from all experiments, change the **Current experiment** option to **All experiments**. Click on the arrow button again if you want to hide the display options from view.

## Other Profile Measures

Other useful measures are available through the *Measures selections* option in the *Sample* menu. Measures contained in the **Profile** group include:

**G/ Dens** – Graph Density. Mean density value of the sampled region.

**G/ DxA** – Graph Density x Area. Graph Density (G/ Dens) multiplied by its corresponding image area.

**G/ Peak** – Peak Density. Maximum density value of sampled region.

**G/ Pos** – Graph Position. Current position, taken from the beginning of the line. If a section of the line has been sampled, the value refers to the midpoint of the region of interest.

Distance is calibrated to the current spatial unit (see *Chapter 7* in the online *Reference Manual* for more details about spatial calibration).

**G/ wPos** – Weighted Graph Position. Position of the region of interest, weighted toward higher density values.

**G/ Rp** – Relative Graph Position. Graph Position (G/Pos), expressed as a proportion of the entire length of the profile.

**G/ wRp** – Weighted Relative Graph Position. Weighted Graph Position (G/wPos), expressed as a proportion of the entire length of the profile.

**G/ pPos** – Peak Position. Position of maximum density value of sampled region.

**G/ Width** – Graph Width. Width of the region of interest on the profile. If a single point has been sampled, width = 0.

**G/ Area** – Graph Area under the profile. Derived by multiplying Graph Width (G/Width) by Graph Density (G/Dens). **NOTE:** Do not use this measure with nonlinear spatial calibrations (e.g., molecular weight).

**G/ DxA (%Total)** – Graph Density x Area (G/ DxA), expressed as a percentage of the total area scanned.

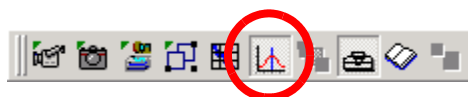
**G/ Dens (%Peak)** – Graph Density value of sampled line point(s), expressed as a percentage of the highest single density value on the profile.

**G/ Area (% Total)** – Graph Area under the profile (G/Area), expressed as a percentage of the area under the entire density profile.

## Direct Band Analysis: Basics

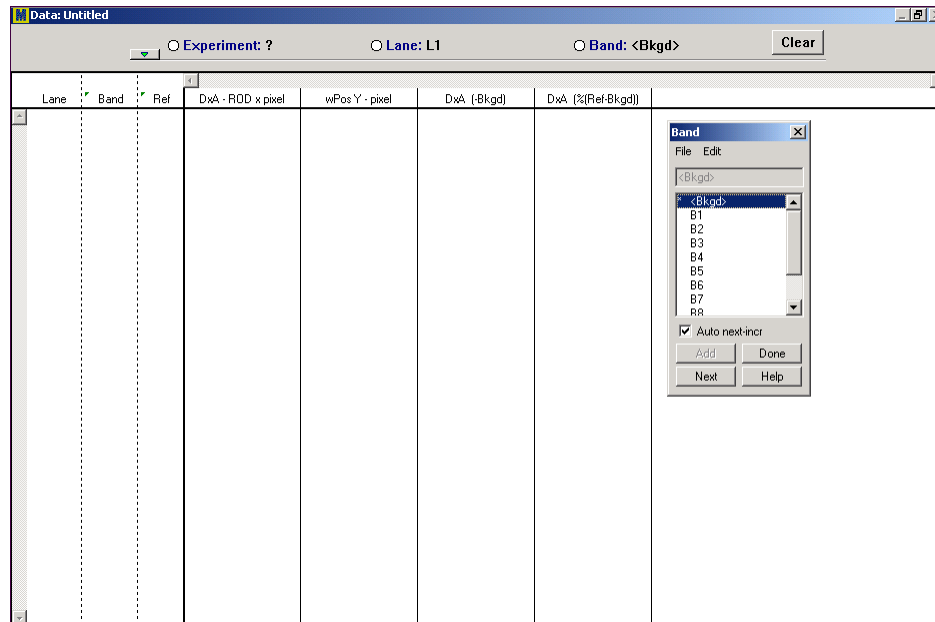
In “Direct band analysis” mode, **MCID Basic** is configured to gather data directly from the image, without obtaining a density profile. Direct measures include position and integrated area (i.e., density x area).

When you select the **Gels: Lane Analysis** study type, **MCID Basic** is configured, by default, to generate a density profile. If you want to perform direct band analysis, de-select the **Profile** icon located in the **Application** toolbar:



MCID Basic then loads the appropriate measures for direct band analysis.

**Figure 19:** The MCID Basic Sample window, configured for direct band analysis.



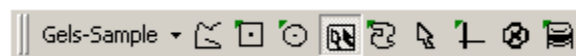
Direct band analysis involves the following general steps:

- Digitize the specimen.
- Perform all necessary calibrations.
- Establish a background density value for a given lane.
- Sample the band(s) contained within the lane.
- Repeat steps 3 and 4 until all bands have been sampled.

Data are reported to the data table, with background density automatically subtracted from the band data.

## Sample Tools

**Gels-Sample** tools (*View > Image Tools Bar*) are used to establish a background density value and to gather data from individual bands:



The **Gels-Sample** tools and their functions are listed below.

1. The **Outline** tool is used to trace around an area of interest.
2. The **Box** tool's size and orientation can be adjusted.
3. The **Circle** tool's size and orientation can be adjusted.
4. The **Autoscan** tool creates outlines automatically.
5. The **Template** tool creates user-defined templates.
6. The **Pointer** tool selects data from a sample window.

7. The **Pixel info** tool gives the X, Y, and the density value for individual image pixels.
8. The **Delete** tool deletes sample windows from the image and any associated data.
9. The **Eraser** tool erases image overlays.

Each of these tools is explained in greater detail in the online *Reference Manual (Chapter 4: Collecting Data)*.

### Establishing a Background Density Value

In order to obtain accurate density measures for a specific band, you must subtract the irrelevant density levels contained within its lane. **MCID Basic** will subtract this value automatically once the background density value has been established. The background density value is established by sampling a region of film or plate background between bands. Ideally, the region should be representative of the background density that is present throughout the entire lane.

To establish a background density for a given lane, set the current “Band” label (located just above the data table) to <Bkgd> (see “Labeling Data” below). Use any **Gels-Sample** tool to sample a representative region of lane background.

### Gathering Data

Set the current band label to “B1”. Select a **Gels-Sample** tool and use it to sample each band in the lane. **MCID Basic** will automatically assign a “band” label (B1, B2, B3, etc.) to each band as it is sampled, in consecutive order. Data relating to the sampled area will appear in the data columns.

The following default measures appear in the data table:

**D x A** – Mean density of the sample window, multiplied by its area.

**wPos Y** – Position of sample in Y axis, weighted toward higher density values.

**D x A (-Bkgd)** – Lane background density is subtracted from the density of the sample window, then multiplied by the area of the area of the sample window. This measure may be used as an estimate of above-baseline signal intensity.

**D x A (% Ref - Bkgd)** – D x A, expressed as a proportion of the D x A value of a reference band. Lane background density is subtracted from both the sample and the reference band. See “Reference Data” below.

The units in which these measures are expressed depend on whether the image is calibrated or not. If the image is not calibrated, density measures are expressed as “relative optical density (ROD)”. Spatial parameters (i.e., position, area) are expressed in pixels. See the [Calibrations](#) section of this document for information about image calibration.

To select other measures, open the *Sample* menu and select the *Measures selection* option.

To gather data from another lane, select a new lane label (e.g., L2), establish a new background value for the lane, and sample the bands.

## Labeling Data

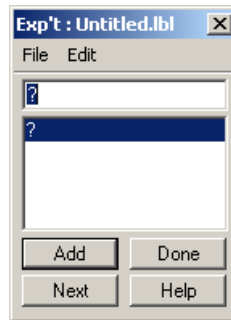
MCID Basic tracks gel data by **Experiment**, **Lane**, and **Band** labels, located at the top of the Sample window. You can use the default MCID Basic labeling scheme or create your own labels.

To view a list of labels, click on the label heading at the top of the screen. A dialog box will appear for the selected label type. Each label dialog box contains a list of labels and an entry field for typing new labels. The label dialog box remains in place until you press **[Done]**.

### Experiment Labels

**Experiment** labels are used to designate individual gels or images. The default label is “?”. To change the default label, click on the **Experiment** heading at the top of the **Sample** window. When the *Experiment* dialog box appears place the cursor in the label entry field and type the new label name. This becomes the current experiment label and is added to the label list.

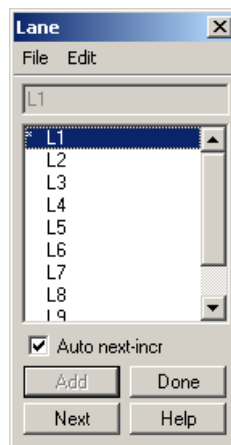
*Figure 20:* The Experiment label dialog box. To create a new experiment label, type the label in the entry field and press the “Add” button.



### Lane Labels

Lane labels are used to designate individual lanes. By default, MCID Basic designates each lane as L1, L2, etc. To view the label list, click on the **Lane** heading at the top of the screen. A *Lane* label dialog box appears.

*Figure 21:* The Lane label dialog box, showing default MCID Basic lane labels.



To select a new lane label (i.e., when you are ready to gather data from another lane), highlight the label in the list. The selected label becomes the “current” lane label and the band label automatically re-sets to “<Bkgd>”.

### Band Labels

**Band** labels are used to designate individual bands or spots. To view the band label list, click on the **Band** heading at the top of the screen. The *Band* label dialog box will appear.

By default, **MCID Basic** automatically assigns a label (B1, B2, etc.) to each band as it is sampled, in consecutive order. This means that you do not have to select a new label each time you sample. If the *Band* label dialog box is in view, the current label is highlighted and an asterisk marks the next label that will be used.

The **Band** label dialog box also contains a “<Bkgd>” label for lane background density. Be sure that this label is selected when you establish a background density value for a given lane. When you select a new lane label (i.e., when you are ready to gather data from another lane), the band label will automatically re-set to “<Bkgd>”. Once the background value is established, the band label will change to “B1”.

**Figure 22:** The *Band* label dialog box, showing the MCID Basic default labeling scheme.



### Reference Data

The **D x A (-Bkgd)** measure for a given band can be expressed relative to data from a “reference” band in the same lane. In the data table, this measure is represented as **D x A (% (Ref-Bkgd))** and is expressed as a percentage.

Any band in a lane can be used as a reference. To designate a band as a reference, double-click in the reference (**Ref**) column beside that band’s data. The reference column is the narrow column between the labels at the left and the numerical data at the right. To deactivate a reference, double-click in it again.

Please note that the reference band must be in the same lane as the bands of interest. If you want to compare the signal intensity of bands in different lanes, you must sample them under the same lane label.

## Direct Band Analysis: Advanced Details

### Creating Your Own Lane or Band Labels

To clear the default labeling scheme and create your own labels, select *File > New* from menu within the dialog box. The label list now contains a single “?” as a label. To enter a new label, place the cursor in the label entry field and type the new label name. This name becomes the current lane or band label and is added to the label list.

For example, suppose you wish to replace the default lane labels with labels that reflect specific cell lines (e.g., Att20, GH3, P19 and Cos-7):

- Click on the **Lane** label at the top of the **Sample** window. The *Lane* label dialog box appears with the default lane label list.
- Open the *File* menu on the *Lane* label dialog box and select *New*.
- Enter the label “Att20”
- Establish a background density for the lane and sample each band in the lane.
- Repeat steps 3 and 4 (using the appropriate lane labels) until all lanes have been sampled.

You do not have to retype a label once it is present in the list. Simply click on it to make it the currently active label.

You can create your own band labels in the same way. The “<Bkgd>” label, however, will always remain as the first label in any list that you create. Turn on the **Auto next-increment** option if you want **MCID Basic** to select the next label in the list each time you sample.

### Saving and Editing Label Lists

Label lists can be saved to disk, retrieved, and edited. The *File* menu in the label box allows the usual file manipulations of the label list (new/open/save/save as). Label list files are stored with an “\*.lbl” extension. Because all label list files have the same extension, *do not save a Lane label list with the same name that you gave to a Band list.*

To edit label lists use the *Edit* menu.

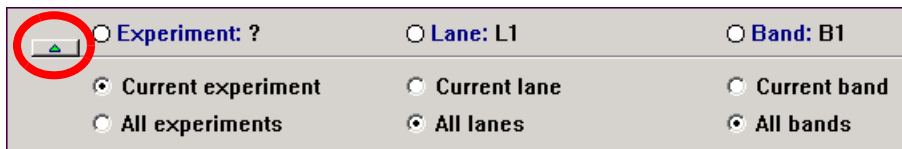
- To delete the selected label use *Delete*.
- To change the selected label use *Change*. For example, we might want to change an experiment label of “Gel 10” to “Gel ten”.

### Data Display Options

By default, the Sample window displays data gathered from all bands in all lanes from one experiment (i.e., all data obtained from one gel). This is the **Basic** data display for gel analysis. If you have gathered data under different Experiment labels, you must select the appropriate Experiment label in order to view its data. You can use the **Extended** data display to change the display configuration, however, so that data from all experiments are displayed in the data table.

Options for **Extended** data displays are located under the Experiment, Lane, and Band headings at the top of the screen. By default, these display options are hidden from view. To

switch between the **Basic** and the **Extended** data displays, click on the arrow button to the left of the **Experiment** heading.:



The **Extended** data display controls allow selection of one or more experiments, lanes, and bands. If you wish to display data from all experiments, change the **Current experiment** option to **All experiments**. Click on the arrow button again if you wish to hide the display options from view.

## Other Measures

Other useful measures are available through the *Measures selections* option in the *Sample* menu.

**Dens** – Integrated optical density (mean density value of all the pixels in the sample). Select this measure from the “Density” group of measures.

**Area** – Area of the sample. Select this measure from the “Basic Morphometry” group.

**wPos X** – Position of sample in the X axis, weighted toward higher density values. Select this measure from the “Position” group.

Several other “background corrected” and “reference” measures are included in the “User Defined” group of measures. Please see *Chapter 5: Selecting Measurements* in the online *Reference Manual* for details.

## Calibrations

If the image is from an internally calibrated device (e.g., a phosphor imager or scanning densitometer), **MCID Basic** shows the density and spatial units from that device. Because most of these devices are linear over a broad dynamic range, density calibration may be unnecessary.

If the image is from an uncalibrated device, such as a video camera, the **MCID Basic** data columns are also uncalibrated. Densities are expressed as Relative Optical Density (ROD) and spatial measurements are expressed in pixels. Gels tend to exhibit a rather broad range of densities and it is probable that a camera is responding non-linearly over some portion of that range. Calibration to a density step tablet will help provide linear density values.

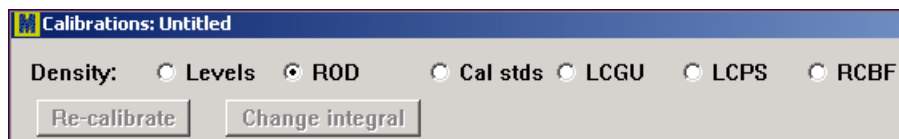
Calibration to external standards is discussed in detail in the online *Reference Manual* (*Chapter 7: Calibrating Images*).

## Uncalibrated and Calibrated Images

In uncalibrated image acquisition (from cameras), **MCID Basic** uses relative optical densities (ROD) or gray levels (Levels). Use ROD for specimens with dark peaks. Use gray levels for specimens with bright peaks (e.g. UV gels). To change either of these density units, go to the **Calibration** operation window and select the *Density* option from the *Calibrations* menu.

When the *Density Calibration* window appears, select the appropriate density unit (**Levels** or **ROD**) from those displayed at the top of the screen.

**Figure 23:** To change the current uncalibrated density unit, go to the density calibration window and change “ROD” to “Levels”.



When acquiring images from a calibrated scanner, the density measurement unit and the spatial calibration are supplied by the scanner. A brief summary follows.

### Fujix BAS

The density unit is “photo-stimulated luminescence / unit area” (PSL/mm<sup>2</sup>), an internal calibration used by the BAS scanner family. “PSL” is linearly related to dpm in the specimen.

### Molecular Dynamics Personal Densitometer™ or PhosphorImager™

The Personal Densitometer is internally calibrated to optical density. The density unit will be “OD”. The PhosphorImager is internally calibrated to “Counts” / unit area, which is analogous to Fujix BAS “PSL/mm<sup>2</sup>”. The **MCID Basic** abbreviation for Molecular Dynamics “count” is “MDC”.

### Bio-Rad® Molecular Imager®

Bio-Rad’s phosphor plate imager is internally calibrated to “Counts” / unit area. The **MCID Basic** abbreviation for Bio-Rad “counts” is “BRC”.

### PerkinElmer® Cyclone™

The PerkinElmer (Packard) Cyclone scanner is internally calibrated to Molecular Dynamics “Counts” / unit area. The **MCID Basic** abbreviation for Packard “counts” is “MDC” (Molecular Dynamics Counts).

## Density Calibration: The Step Tablet

The step tablet (also called a “step wedge”) is a thin strip of film coated with about 20 grayscale “steps”. It is accompanied by a sheet of paper (from Eastman Kodak®) that lists the “Diffuse Density” value for each step. The calibration procedure is the same as that used for calibrating to concentration standards, described in detail in *Chapter 7: Calibrating Images*. The general procedure is as follows:

1. Adjust the camera height to the position it will be at when gathering data from the specimen.
2. Perform a **Flat Field Correction** (see *Chapter 2: Acquiring Images* in the online *Reference Manual*).
3. Digitize the step tablet. Adjust the illumination so that the clear portion of the tablet is pinkish-blue when viewed with the SPECT2.VIS or SPECTRAL.VIS look-up tables.

4. Change the **Standards Unit** to “OD” (optical density).
5. Enter the “Diffuse Density” values into the **Std Value** column of the calibration table (or load the values from a standards file, if you have one). Use only the first 10 or 12 values.
6. Starting with the lightest standard, sample each step in ascending order. Re-digitize and re-position the step wedge as needed, but do not change the camera height. Please note that the first “Diffuse Density” value (0.04 - 0.05) corresponds with the clear portion of the step tablet.
7. Select a curve fitting function.
8. Select an extrapolation function.

Once you have established the calibration, return to the **Sample** window and digitize your specimen. Re-calibrate if the magnification and/or illumination level is changed.

### Spatial Calibrations

As with density, **MCID Basic** accepts the spatial calibrations provided by calibrated instruments such as phosphor imagers.

If you are working with uncalibrated images, you are free to calibrate the spatial dimension(s) to any distance unit or to leave distance in uncalibrated pixels. For many gel applications, spatial calibration is unnecessary.

If it is useful to know a molecular weight of a spot or band, you must establish a **Position** calibration. **Position** calibration requires that you establish the horizontal (or vertical) pixel coordinates for a number of reference marks contained in the image (e.g., molecular weight markers). A mathematical function is fit to the points and is then used to interpolate values between them.

### Position

Open the *Calibrations* menu and select the *Position* option. Select the axis you wish to calibrate and change the Position unit from **Relative** to **Calibrated**. You may calibrate in either the X or the Y axis. When **Calibrated** is clicked, **MCID Basic** requests the name of a file that contains the standard values (i.e., the molecular weight values). Click **[None]** if you do not have the values on file.

The **Calibration** window now looks much like it does when you establish a **Density** calibration. On the left side of the window is a table with four columns labeled **Std #**, **Std Value**, **Value Read**, and **Rel Error**. Just above the table is an entry field that contains the **Standards** unit. To the right of the table is the region where the calibration curve (**pixel coordinate** versus **standard value**) are plotted.

If you are using a standards file, the standard values are displayed in the **Std Value** column. Otherwise, type the values of the standards into this column and enter the appropriate units (e.g., “Kb”) into the **Standards unit** entry field. When you have entered all of the standard values, click the cursor in the **Value Read** column next to the first standard value.

Adjust the camera height so that you can see the reference markers. Move the cursor to the Image View. If you are calibrating the Y axis, the cursor turns into a horizontal line that moves up or down as you move the mouse. Align the line with the middle of the first (uppermost) standard and click the left mouse button. The pixel position (Y axis coordinate)

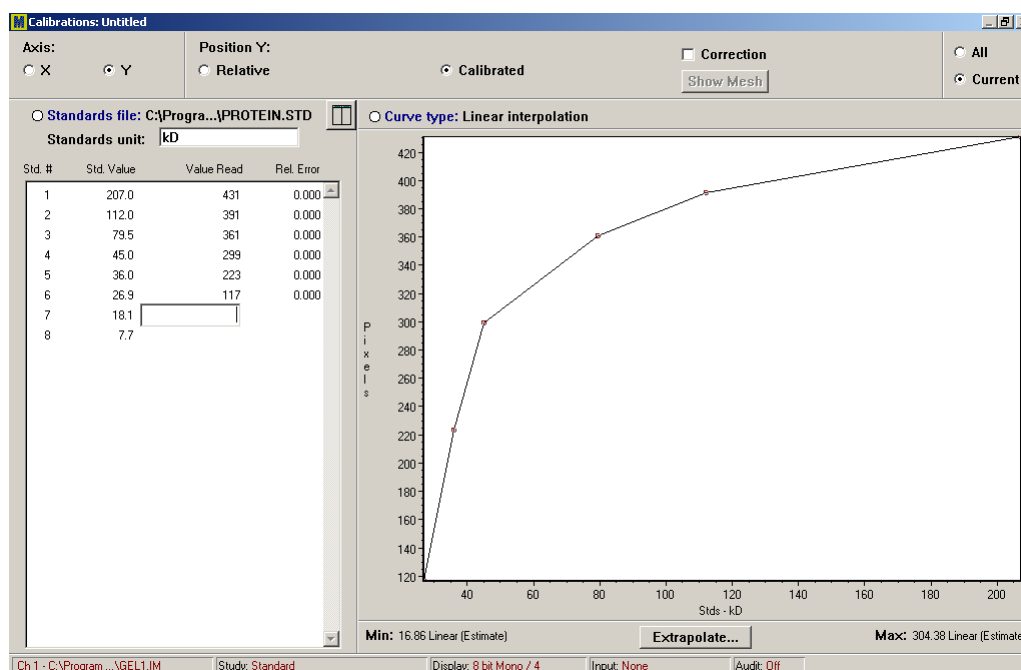
appears in the **Value Read** column and the entry field automatically moves into position for the next standard. Repeat the above procedure for the remaining standards.

Once you have constructed the calibration table, you need to fit a curve to your data points. The curve fitting function that you select will be used to interpolate a calibrated value (e.g., Kb) for each pixel from its position in the Y axis. By default, **MCID Basic** draws straight lines between the individual data points (linear interpolation). To access other curve-fitting functions, click on the **Curve type** button at the top of the graph area.

### *Editing or Generating a New Calibration*

To edit a standard value, highlight it and retype the value. To re-position a reference line, place the cursor over the line until it becomes a double-arrow. Then drag the line to re-position it. To create a new calibration table, click on **Standards file**. **MCID Basic** clears the existing calibration and is ready to receive a new one.

**Figure 24:** Example of a Position calibration to molecular weight standards.



### *Correcting for Curvature*

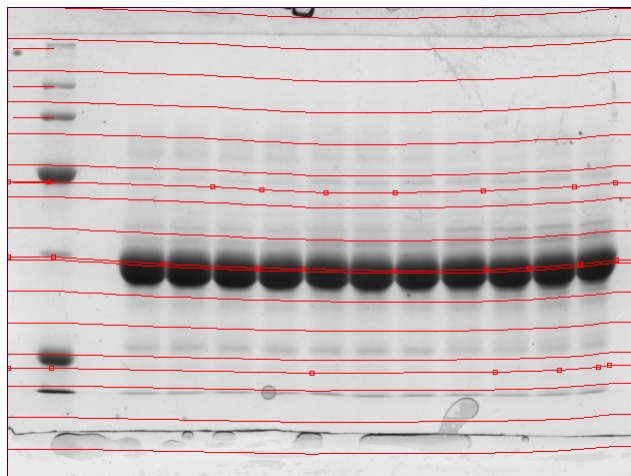
Gels frequently exhibit spatial distortions that could affect position measures. For example, if all of the bands on the right side of a gel are skewed upwards, then the molecular weight measures obtained from these band would be artifactually high. To overcome this problem, you can re-establish the molecular weight boundaries by clicking on points *across* the gel.

1. Click on the **Correction** checkbox at the top of the **Calibration** window.
2. Click on the first molecular weight standard to “anchor” the cursor.
3. Move the cursor to the right, clicking on points that follow the curvature of the gel. Double-click on the right-most point.
4. Repeat the procedure for each molecular weight standard.

5. Press [**Show Mesh**] to see how **MCID Basic** will interpolate molecular weight values in between each of the markers (Figure 25).

The molecular weight calibration is now corrected for curvature.

**Figure 25:** A molecular weight calibration corrected for curvature.



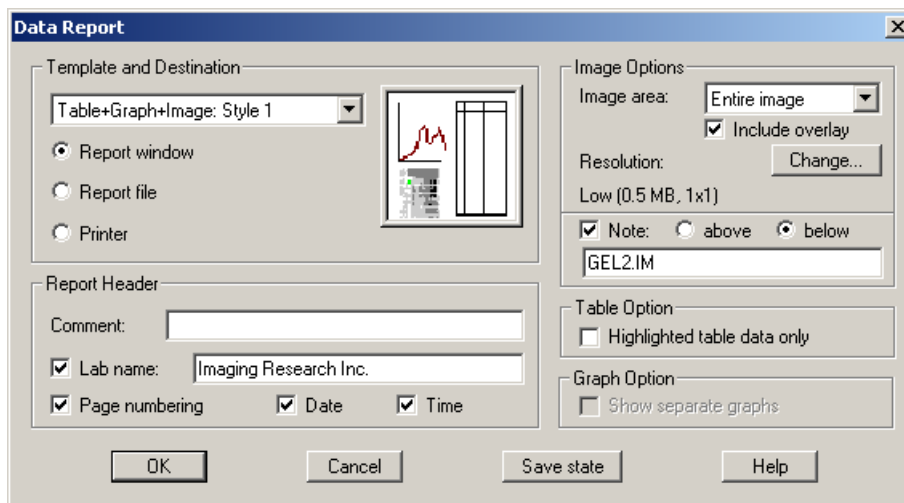
## Creating Custom Data Reports

“Data reports” are a convenient way to summarize the results of a gel analysis session. With a single command, you can save or print an image, density profiles, and numerical data from the profiles as a unified report file.

Data reports do not replace log files. In contrast to the log file, the data report does not contain any calibrations and it cannot be edited at a later date. We recommend that you save your results in log files and use the data report function to summarize data for placing into notebooks.

To create a report, select *Sample > Data report*. This displays a *Data Report* dialog box.

**Figure 26:** The *Data Report* dialog box.



### *Template and Destination*

The contents of the report are controlled by selecting a template. Some templates contain a data table, graph, and image. For example, the “Table+Graph+Image: Style 1” option generates a graph above an image, with a data table on the right side of the report. Other templates arrange these components differently or contain only some of these components. If you have performed direct band analysis, select one of the “Table+Image” templates. The preview box shows how the report will be organized.

You can direct the data report to the following destinations:

**Report window** – Data report is directed to the **Report** operation window. You can view, print, export (as a text, Lotus, Excel or TIFF file), or save a report file to disk.

**Report file** – Data report is saved directly to disk as a report file. **MCID Basic** automatically attaches an “.rep” extension to the filename.

**Printer** – Data report is printed.

### *Image Options*

The image in the report may contain just the contents of the Image View or the entire image that extends beyond the Image View. Select **Viewed region** to place only the Image View into the report. Select **Entire image** to place the entire image into the report.

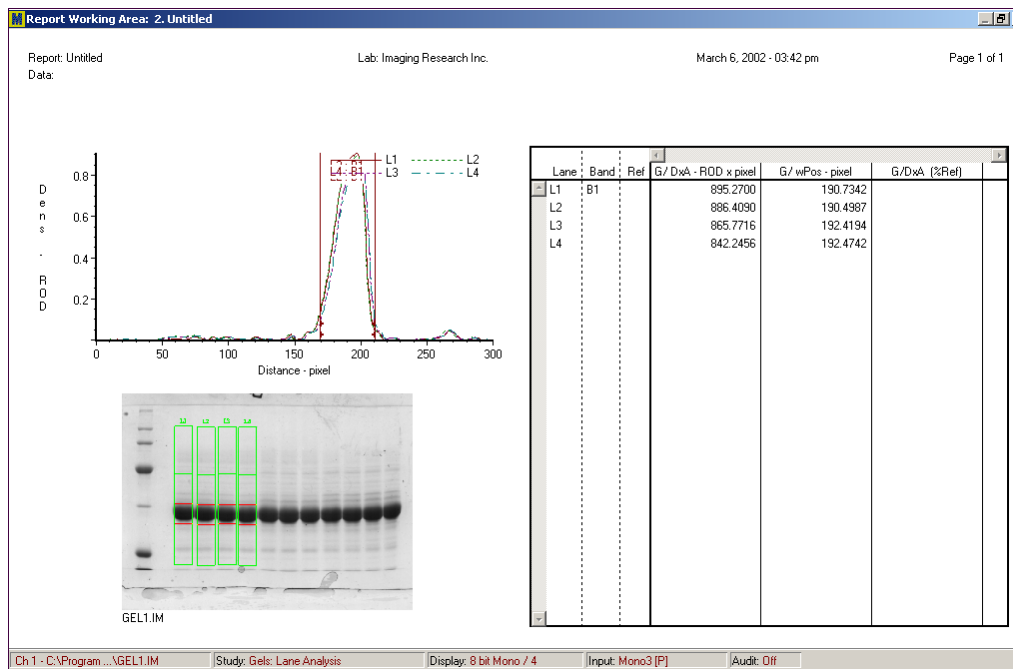
The overlay includes profile outlines, lettering, and any other graphics on the image. You will usually want to keep the overlay report turned on.

Options are also available for manipulating the resolution of the image component of the report and for the positioning of the image filename.

### *Report Header*

Various informational labels may be included with the report, including the laboratory name (i.e., the name of the official **MCID Basic** licensee), page numbers, date, and time. To include any of these labels, click on the corresponding checkbox.

**Figure 27:** The Data Report screen after selecting the “Table+Graph+Image: Style 1” option from the Template section of the Data Report dialog box.



### The Report Window

If you have created a data report and have selected **Report window** as the destination, **MCID Basic** will place you into the **Report** operation window:



From the **Report** window, you can view the report and control how it is printed or saved.

### Report File Functions

The **File** menu in the **Report** window is used to open, print, save, or export data reports:

**Open** – Displays the selected data report file (REP) in the **Report** window.

**Save as** – Saves all components of the **Report** window to a disk file. **MCID Basic** automatically attaches an “\*.rep” extension to whatever filename you enter.

**Print** – Sends the report to the printer.

**Export** – Saves the numerical components of the report (data table and/or profile data points) in either text (\*.txt, \*.csv, \*.prn), Lotus 1-2-3 (\*.wks) or Excel (\*.xls) formats. You can export a page of the report as a graphics file, in TIFF format. See below.

### Exporting a Data Report

From the **Report** window, use the **File > Export** command to export data. When the **Export Report File** dialog box appears, select the desired file format (text, Lotus 1-2-3, Excel or TIFF) and enter a filename. **MCID Basic** attaches the appropriate extension to the filename as it saves the file to disk.

**Note:** You must have Microsoft Excel installed on your computer to be able to export files in Excel format.

If the **Report** window contains a data table, the exported text, Lotus 1-2-3 and Excel files will contain the data display, just as it appears on your monitor. If the report contains a graphical component, the files will contain a column of density values corresponding to each point along the profile, from left to right. Click on the **[Options]** button in the *Export Report File* dialog box if you use larger intervals (e.g., every tenth data point).

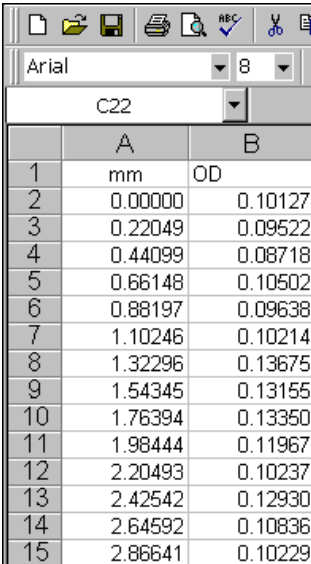
If there are multiple profiles, you can export the data values for each profile, one after the other. If the report contains a data table *and* a graphical component(s), **MCID Basic** includes all datasets in the exported file.

If you select TIFF as the export file type, **MCID Basic** formats a current page of the active report as a medium-resolution color graphics file.

### Text File Formats

The different text file formats dictate how columns of data are separated from each other. Data may be separated (delimited) by tab stops (\*.txt), by commas (\*.csv), or by spaces (\*.prn). Select whichever format is appropriate for the software that will read the exported file.

**Figure 28:** Part of an Excel spreadsheet containing data exported from an MCID Basic profile. Distance along the X axis is in the first column. The second column contains density values.



	A	B
1	mm	OD
2	0.00000	0.10127
3	0.22049	0.09522
4	0.44099	0.08718
5	0.66148	0.10502
6	0.88197	0.09638
7	1.10246	0.10214
8	1.32296	0.13675
9	1.54345	0.13155
10	1.76394	0.13350
11	1.98444	0.11967
12	2.20493	0.10237
13	2.42542	0.12930
14	2.64592	0.10836
15	2.86641	0.10229

**TRADEMARK INFORMATION**

M3D, MCID, AIS, MCID Analysis, TFM, Imaging Research, and their related logos are either trademarks or registered trademarks of Imaging Research Inc. in Canada, the United States and/or other countries.

Pentium is a trademark or registered trademark of Intel Corporation or its subsidiaries in the United States and/or other countries.

Sony is a registered trademark of Sony Corporation.

Amersham is a trademark of Amersham plc.

Lotus is a trademark of Lotus Development Corporation.

Microscales is a trademark of Amersham Biosciences Limited or its subsidiaries.

SPOT and SPOT RT are trademarks or registered trademarks of Diagnostic Instruments, Inc. in the United States and/or other countries.

Windows & Microsoft is a trademark of Microsoft Corporation in the United States and/or other countries.

CoolSNAP, PVCAM, Cascade, Quantix, SenSys and Photometrics are trademarks or registered trademarks of Roper Scientific, Inc

Bio-Rad, and Molecular Imager are registered trademarks of Bio-Rad Laboratories, Inc.

Kodak is either a registered trademark or trademark of Eastman Kodak Company.

PerkinElmer and Cyclone are either registered trademarks or trademarks of PerkinElmer, Inc. or its subsidiaries in the United States and/or other countries.

Personal Densitometer, and PhosphorImager are trademarks of Amersham Biosciences Limited or its subsidiaries.

All other names are trademarks of their respective companies.

Copyright © Imaging Research Inc. 2003 - All rights reserved.

**InterFocus Imaging Ltd  
Cambridge Road  
Linton  
CB1 6NN  
England**

**TEL: +44 (0)1223 894833 – FAX: +44 (0)1223 894235**

**US & Canada Toll Free: 1-866-782-2202 – Fax: 1-917-591-9130**

**Email: [sales@mcid.co.uk](mailto:sales@mcid.co.uk)**

**Visit MCID Online at [www.mcid.co.uk](http://www.mcid.co.uk)**