94 1068

# Determining the Noise Segment Used in the Spectral Contrast Technique

Steve Harrington

The Waters 996 Photodiode Array Detector (PDA) together with Millennium software uses a complex mathematical technique to automatically determine spectral library matches of standard to sample spectra and compound purity testing. This complex algorithm determines the differences in spectral shape by converting the overall shape of spectra to vectors in multi-dimensional space and then comparing those vectors.

Millennium® software stores standard spectra within a spectral library and unknown spectra are compared against those standards. In peak purity, the spectrum at the peak apex is the reference spectrum. All other spectral data contained within that peak are compared to the spectra of the apex.

Match results and purity results are reported in degrees. If two vectors (representing spectra) overlap completely, there is a difference of zero degrees between them; therefore, they are the same. If the angle between the two spectra is greater than zero degrees, differences in spectral shapes exist. If the two spectra do not overlap, the angle difference between the two vectors representing the spectra is ninety degrees; therefore, the Match and Purity angles would be between zero and ninety degrees.

Spectral Contrast also reports a second number in degrees: Purity Threshold for peak purity and Match Threshold for spectral library matches (see Figure 1). What are these threshold angles? Purity Threshold and Match
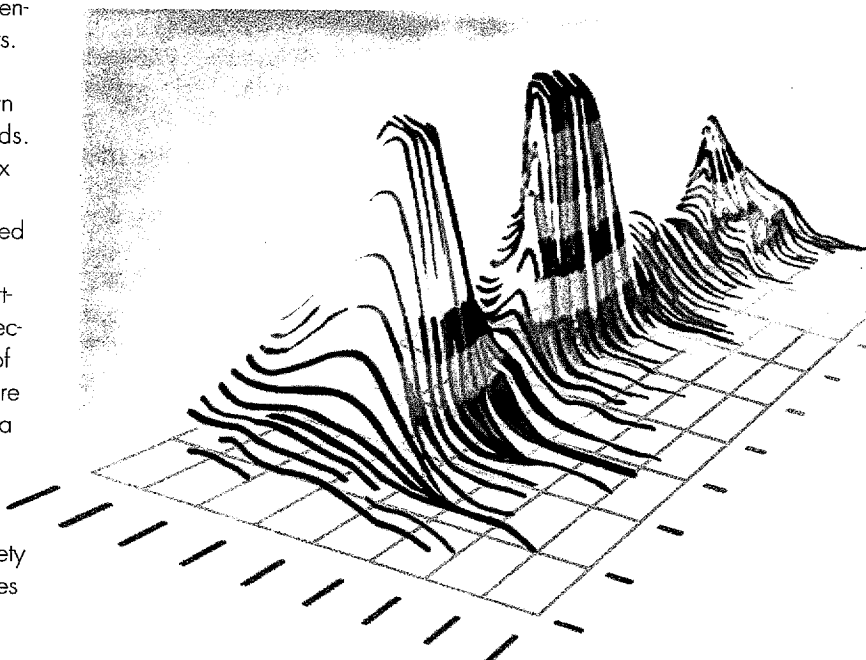
Waters

Figure 1

Figure 2
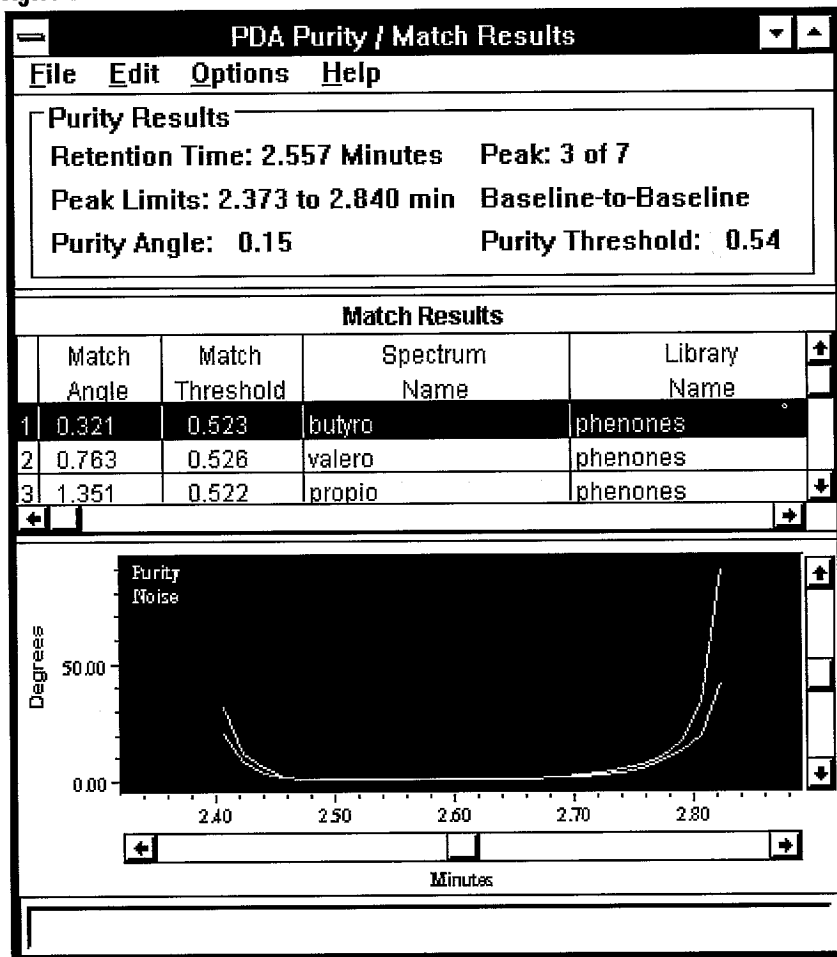
Threshold are based on a calculation of statistical and thermal variations of the absorbence measurements made by the 996 PDA detector. By using the Peak Purity algorithm to compare these values, you can judge how spectrally homogenous a component is. You can also determine how well a sample component's spectrum matches the standard spectrum contained in the library. When there is a spectral shape difference between spectra of the same compound within the same vial, the difference can be the result of noise.

How can you tell the difference? A simple guideline to follow is that if the Purity Threshold is greater than the Purity Angle, the component is spectrally homogenous. The same rule applies for spectral library matching: If the Match Threshold is greater than the Match Angle, it is a good match to the standard spectrum and you can consider it to be the same compound. If the Purity or Match Angles are greater than the Threshold Angles, actual spectral differences exist beyond the contribution of noise.

How can you calculate noise? The noise calculation is based on a segment of baseline within the chromatographic run. This segment does not contain any chromatographic peaks or chromophores beyond the mobile phase background across the specified wavelength range (see Figure 2). The length of the baseline must be two to four peak widths at half height of the narrowest peak of interest within the chromatographic run. It should also contain 20 to 40 spectral data points. For example, suppose you were to collect data at an acquisition rate of one spectra per second (chosen within the 996 PDA instrument method), and suppose that the width at half height of the smallest peak of interest was calculated to be 10 seconds. To determine noise or threshold, the length of the baseline should be 20 to 40 seconds. This segment of baseline is used to determine both the Purity Threshold for the Peak Purity algorithm and the Match Threshold for the Spectral Library Search Algorithm (See Figure 1).

The easiest way to determine which segment of baseline you should select is to create a MaxPlot Channel. To do this, select Derived Channel from the Edit menu in PDA Review (see Figure 3). In the first channel box, select PDA 3D. Then click on the down arrow and select MaxPlot. Enter the same start and end wavelengths (see Figure 4) that you specified in the Spectral Contrast portion of the PDA processing method shown in Figure 1.
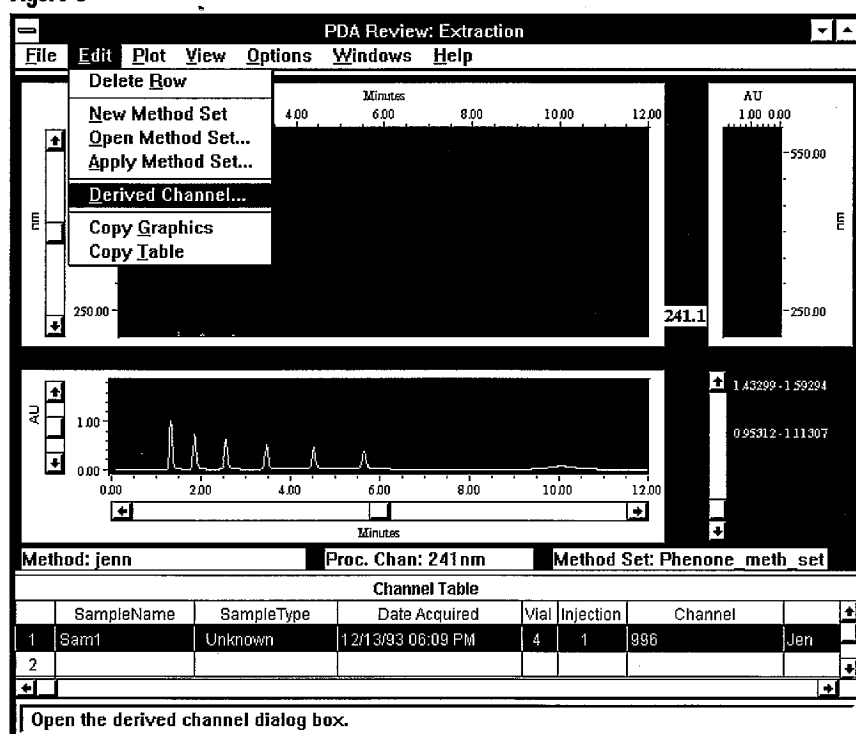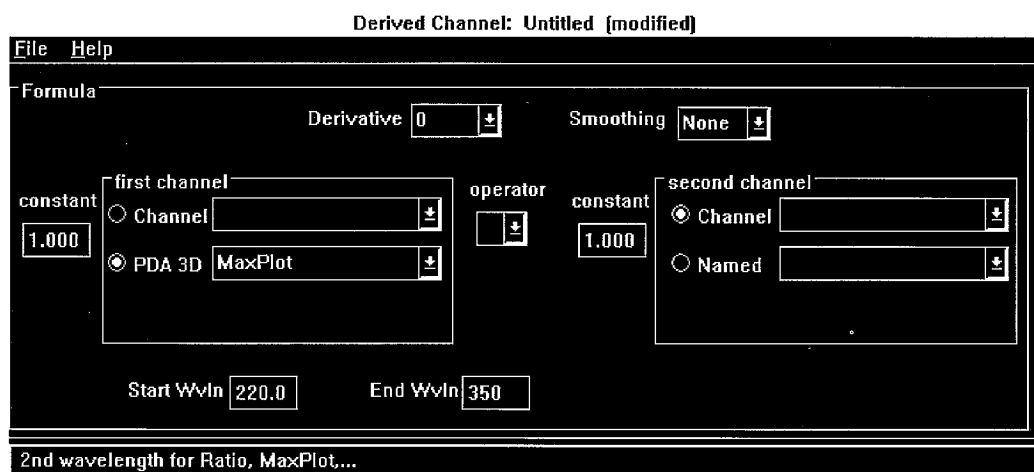
Figure 3

Figure 4

After you finish the extraction parameters, save the MaxPlot Channel by selecting Save As from the File pull-down menu, as shown in Figure 4. (The Save As/File menu does not appear in Figure 4.) Once you enter a new name for the channel, the MaxPlot will automatically be extracted in the chromatogram display of the PDA Review. You can then use the mouse to select and zoom in on a segment of baseline in the extracted MaxPlot chromatogram (see Figure 5) that does not contain chromatographic peaks. In this way, the time

segment you enter can be used in the Noise Interval Time parameter of the Spectral Contrast portion of the PDA processing method illustrated in Figure 1. You can also zoom in on a segment of baseline to verify your choice by selecting the 3D Plot option under the Windows pull-down menu of PDA Review.

The reason for selecting MaxPlot to determine which location to use for the noise segment is that the data points used to create this

Figure 5

chromatographic channel are the maximum absorbence of the spectrum acquired at that point in time. In other words, the maximum absorbence data point is chosen from all the wavelengths within the range that was entered in the derived channel (see Figure 4). Any compound that may be present and that absorbs in the specified range can be seen chromatographically. You can also use the 3D plot option to confirm if any drift is present within the wavelengths of the specified range that may adversely affect the noise (threshold) calculation.

Spectrum Review is another tool within Millennium software that allows you to examine the Noise Spectrum under the Plot pull-down menu. You can use Spectrum Review to verify if any component spectra may have been included in the baseline segment you selected. Note that you must first process the data using the PDA processing method before the noise spectrum is accessible. If you have any questions about your Library Match or Peak Purity results, use the 3D Plot option and Spectrum Review to help you troubleshoot them and determine the baseline noise segment.
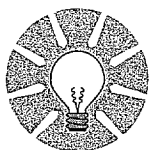
IDEAS THAT WORK...

Making the Best Use of Demo and Default Projects

Every newly installed Millennium workstation includes the projects, Demo and Default. The Default project contains a number of templates that you can use when you are developing methods for your laboratory. Your Millennium software also includes samples of GPC, PDA and System Suitability Report methods as well as a small assortment of other types of methods.

When you create new projects, you have the option of copying methods and filters from already existing projects. If you decide to do this, information is copied from the Default project unless you specify a different source. Note that if you make an error while you are modifying any of the templates in the Default project, that error will be copied into all new projects. The Default project cannot be deleted.

The Demo project contains a collection of data as well as methods. These were designed to help you learn new Millennium concepts. You may also find them helpful when you are troubleshooting problems with data or methods in other projects. For example, if you encounter problems reprocessing when you are using customized reports or newly acquired data, try copying the methods or data from the Demo project. Use them to test the suspect project information. If you need to identify subtle problems in newly developed methods, this approach takes a lot less time than testing every parameter in suspect methods.

ON TARGET

We designed *on-line* with you in mind.
Now we need your input to tell us how we are doing. Were the articles presented of interest to you? What topics would you like to see covered in the future? Or maybe you have an article or success story discussing applications for the Millennium Chromatography Manager.

Either way, we want to hear from you.
Call us at 508/478-2000 and ask for either Judith Rossi (x3602) or Bill Thompson (x2852) or fax us at 508/478-5839, or call your local subsidiary.

Questions and Answers

How can I install my Millennium 2.0 update on multiple drives so I can get better use of my hard disk space?

If you had a previous version of Millennium installed already, you should first do a complete Database Backup. You will use that backup later to include your existing data.

When you install the Millennium 2.0 update, you should choose the option to reinstall (not update) the software. You will have an option at this point to split the installation up on different drives, with designations for Data (raw data), Program files (the Millennium program), and Database files (the Oracle relational database). Then, after the 2.0 installation is complete, restore the old database so that your old data will be included.

The software won't look any different if it is installed on multiple drives, but the benefit is that you will have more total disk space for your data.

What option should I use if I only want to update my Millennium program files to version 2.0?

It is a good idea to back up your Database, or you can also back up individual projects of data, before updating the software. If there is a problem during installation, you could then recover your data by restoring it later.

Install the Millennium 2.0 software by running the Setup program on Disk 1. You will be asked a series of questions about your update, and Setup will check your computer system's resources to make sure that they are adequate to run Millennium. You will be asked whether you want to reinstall or update the software. Choose the update option, which will then ask you if you want to update the Program files only, or to update the Program and Database files. You should always update only the Program files if you want to keep the data that you have already collected, because updating your existing program files will not affect your database data. However, if you update the Program and Database files, you will lose access to the data you acquired in older versions of Millennium.

I enabled Purity in my PDA processing method, processed a channel, and some of my peaks still did not display the purity result. Why is this and how can I get the purity plot for my peaks?

To successfully get peak purity calculated for a peak, you must have the following items set correctly:

1. A peak must be integrated and have at least 10 data points (spectra) across the peak for peak purity to be calculated. If the peak has <10 points across the peak, no purity will be calculated and a message will be displayed in the message board stating "Insufficient Peak Spectra".

2. The noise interval time set in the PDA processing method must be at least 10 data points long (recommended minimum is 30 seconds). If the noise interval has less than 10 data points, no purity will be calculated and a message will be displayed in the message board stating "Insufficient Noise Spectra".

3. The collected wavelength range (set in the instrument method) must have at least 10 times the resolution. If the wavelength range is less than 10 times the resolution, no purity will be calculated and a message will be displayed in the message board stating "Insufficient Wavelength Elements".

How can I get my COLORADO backup Save-Set to restore properly. When I enter the Save-Set name as requested, it will not complete the restore.

Colorado software requests the Save-Set name but actually wants the Save-Set number of the tape. If you have backed up say five separate projects and you want the third one you backed up, you would simply enter 3 when prompted for the Save-Set name. This is regardless of the label you gave the Save-Set when you backed it up. On Colorado software version 3.01 and earlier you must do an index of the tape from DOS and count the number of the Save-Set you desire. With version 3.01 and later, this index will also give you the Save-Set number with the label. This is a limitation of the Colorado software and can not be changed within Millennium.

Quick Reference
Working in Windows
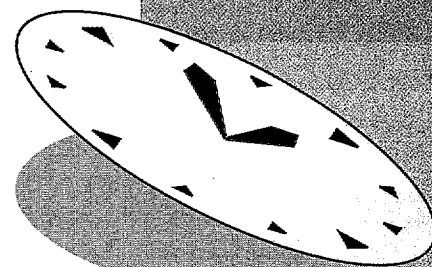
Commands for Windows™ Applications

VIEWING

Command	Result
<CTRL+ESC> or double click on background	Pulls up a Task List that displays a list of all your open windows.
<ALT+ESC>	Pages you through applications that you have opened in the order that you originally opened them.
<ALT+TAB>	Moves through the icons of open applications.

SPECIAL COMMANDS

<ALT+letter>	Executes a command from the menu with the corresponding underlined character. For example: press <ALT+X> for Exit press <ALT+S> for Save.

SELECTING

<CTRL+/>	Allows you to select all items in a list. Useful when selecting all results for reprocessing.
<SHIFT+click left mouse button>	Allows you to select all items from the previous mouse position through the current position. Useful when selecting a sequential range of results to be reprocessed.
<CTRL+click left mouse button>	Allows you to select the current line in addition to any previously selected lines. Useful when selecting non-sequential results to reprocess.

COMMANDS FOR PROGRAM MANAGER

Window Cascade or <SHIFT+F5>	Rearranges open windows, changing sizes and layers so that each title appears.
Window Tile or <SHIFT+F4>	Rearranges and resizes open windows, so that each window appears.

Have you ever opened up different windows or projects on your Millennium workstation and found that you couldn't get to the window you needed to use? Have you ever accidentally clicked on the screen only to find yourself on a different screen? Were you left wondering where the things you were working on went to? Special Microsoft Windows commands make the job of maneuvering around Windows easier.

The tables to the left show you commands you can use within Microsoft Windows applications and commands for Microsoft Windows Program Manager.

Transferring Results and Raw Data To Third-Party Applications Using Dynamic Data Exchange

Keith Wipprecht

Figure 1

	A	B	C	D	E	F
1	SampleName	Vial	Injection	Channel	Processing Method	
2	Std50	3	1	486	parabens	
3	Name	Retention Time	Area	Height	Amount	
4	Uracil	0.492	4915481	880281	1.875	
5	Ethyl	1.742	2288426	288876	0.925	
6	Propyl	3.392	2527448	229472	0.625	
7						
8						
9						
10						
11						
12						

One of the key benefits of Millennium software is that it is based on the Microsoft Windows operating system which offers a variety of ways to share data with other Windows applications. For example, you can copy the result table in the Millennium Review screen to the Windows' Clipboard and paste the results into a spreadsheet application such as Microsoft Excel. Or, use the Copy Graphics menu command to copy a portion of a Millennium screen (such as the chromatogram window) and paste it into a word processing document. Millennium also offers an export method feature that gives you the ability to select a result and export either the result information or the chromatographic data as an ASCII text file.

While each of these methods works well for their intended use, there are some restrictions and limitations inherent in each one. For instance, when you use the copy graphics command to capture a portion of a Millennium screen, the resulting graphic is placed on the clipboard as a bitmapped image. You may want the option to paste this image into a report you're writing and then re-size the picture to conform to your document. However, when you change the size of a bitmap you often distort the original resolution and the result is not always satisfactory.

Millennium software now offers a fully customized way to transfer chromatographic data and results by using the Dynamic Data Exchange (DDE™) interface to the project window. DDE gives you the ability to create chromatograms or photodiode array (PDA) spectral plots at ultra-high resolution that can be placed into a spreadsheet or word processing document and re-sized without the limitations mentioned above. This article will give you an overview of how DDE technology works in Millennium software and what is required on your part to use it.

What Is DDE And How Does It Work?

DDE is a standard protocol established by Microsoft Corporation that allows two Windows applications (such as Millennium and Excel) to communicate with each other so that information or instructions can be passed back and forth to provide a flexible and automated way to exchange data. In order for DDE to work, the software vendor must first build DDE server technology into their product. As of Millennium Version 2.0, the project window utilizes a powerful DDE interface that allows you to use most office productivity applications (Microsoft Excel, Borland Quatro Pro™, or Microsoft Visual Basic) to perform data transfers of either chromatographic results or data using this DDE protocol.

Most major office productivity applications have built-in macro languages which are capable of DDE. This allows you to customize the application so that it can communicate with the Millennium project window. For example, Excel version 5.0 contains the Visual Basic for Applications language (VBA) that gives you the ability to write macros or procedures which perform a sequence of tasks, such as transmitting chromatographic results from Millennium directly into an worksheet using DDE technology. DDE works in a very similar way as a telephone conversation does. You "call" the other application and tell it what you want to talk about, then you send it specific instructions,

such as "send me the results for any chromatograms that are highlighted in the project window." The project window responds by sending the data back to you, and finally you close or "hang up" the conversation.

Each application plays a unique role in the DDE conversation. The application which is requesting the data from Millennium is called the client application. The DDE client application is the one that you customize to request data or other services from the Millennium project window. The project window in Millennium is called a DDE Server because it provides data and other services to the client.

An Example DDE Client Application

What follows is an example of an application using the macro language in Microsoft Excel which provides a turn-key solution to using all of the DDE functions within Millennium. The application provides easy-to-use menus within Excel which allow you the flexibility to export either results or chromatographic data. First you select what result to export in the Millennium project window with the mouse. Next, a dialog box lets you select the chromatographic result parameters to send Area, Height, Amount, etc. Sample header information is included to help identify the result in the third-party application. Figure 1 shows a chromatogram result which has been exported into an Excel spreadsheet using DDE. The format of the result can be fully customized prior to transmitting via DDE from the project window server.

Have you ever needed to incorporate a high-resolution chromatogram or UV spectra from the Waters 996 Photodiode Array detector into a laboratory report? Millennium's DDE capabilities make it easy for you to generate a chromatogram or spectral plot at full resolution for this purpose. Once the chromatographic result is selected in the project window, you just click the command to create an ASCII file of the raw data. You have total control over the format of the chromatographic data as well as the retention time range which lets you transmit all of the raw data or just a subset. Figure 2 shows the 2D Chromatogram DDE Options Dialog that is used to configure the format of the raw data file and the directory it will be sent to.

```
Option Explicit
Dim ChannelID As Integer

        Sub DDE_Chromatogram_Example()

'nitiate a DDE conversation with the DEMO project window
        ChannelID = Application.DDEInitiate("Project", "Demo")

'Include header information for identification purposes
        Application.DDEExecute ChannelID, "[ChromHead(yes)]"

'Include retention time data as well as absorbance values
        Application.DDEExecute ChannelID, "[ChromTimeData(yes)]"

'Include the data for the entire run time of the chromatogram
        Application.DDEExecute ChannelID, "[ChromTimeRange(0.0,end)]"

'Specify the path and file name for the 2D text file, we're going to create a
'file called My2D0000.CH2 in the C:\TEMP directory.
        Application.DDEExecute ChannelID, "[ChromDataFile(C:\TEMP,My2D)]"

'If a file exists with the same name go ahead and overwrite it
        Application.DDEExecute ChannelID, "[ChromFileReplace(yes)]"

'Use a new line character between rows of chromatographic data
        Application.DDEExecute ChannelID, "[ChromRowDelim(\n)]"

'Use a tab character between columns of chromatographic data
        Application.DDEExecute ChannelID, "[ChromColDelim(\t)]"

'Request Millennium to create the data file for any selected 2D result
        Application.DDERequest ChannelID, "CurrentChromsToFile"

'Terminate the DDE conversation with the DEMO project window
        Application.DDETerminate (ChannelID)

        End Sub
```

This example is a macro from Microsoft Excel version 5.0 that demonstrates how to create a text file out of a Millennium 2D chromatogram using DDE. This Sub-Routine creates an ASCII file of a 2D Chromatogram which is selected in the Millennium Project Result View. The DEMO Project is used for this example. (NOTE: For a complete listing of DDE commands that are possible with Millennium software refer to the manual "Using DDE With Millennium Software" which comes with Version 2.0.)

Since the chromatographic file is sent via DDE in an Excel compatible format, all that's necessary to plot the data is to open the file in Excel and select one of the many chart options. You can customize the plot in Excel or any graphics application, copy it to the clipboard, and paste it into your favorite word processing application. Once it's in the word processor you can re-size the plot without losing any of the presentation quality. Figure 3 shows a spectral data plot in Microsoft Word 6.0.

Other Possibilities With Millennium DDE

Millennium software will have additional DDE servers added for Quick Set Control as well as the Result Generator. This will be part of the Millennium Toolkit option which will allow a third-party application such as a Laboratory Information Management System (LIMS) to communicate with Millennium in a seamless way. For example, an operator using a LIMS application can enter a list of samples that need to be run by the HPLC laboratory, and then have that worklist containing sample names and other pertinent information downloaded from LIMS directly to the Quick Set Sample Loading Table in Millennium. When the samples have been run, the chromatographic results can be automatically uploaded back to LIMS if desired. This not only saves the HPLC laboratory time, but reduces the possibility of having manual data entry errors.

The Result Generator DDE server will also allow a result to be sent via DDE to a third-party application which might perform a complex calculation on the result and then return the newly derived result back into Millennium. For example, a result could be sent to a spreadsheet to have a sophisticated statistical calculation performed and then returned to a custom field in the Millennium database. A composite report could then be generated by Millennium which consists of Millennium chromatography results and third-party calculated results — all contained in the same report! This provides some very exciting opportunities for third-party applications vendors to design interfaces between their products and Millennium. At the 1994 Pittsburgh Conference there were several companies marketing their products with Millennium as integrated solutions using the Millennium Toolkit Option.

Summary

Using DDE technology with Millennium software provides you with the power and flexibility to customize how results or chromatographic data are transferred between the Millennium database and applications which support DDE. This opens up all kinds of possibilities for custom tailoring popular office productivity applications so that these applications are "Millennium aware." The benefit is that you can easily incorporate various types of chromatographic information into your company's standardized reports which may be based on a popular office productivity application. Using DDE with Millennium software can be summed up with these words "Now You're Playing With Power!'
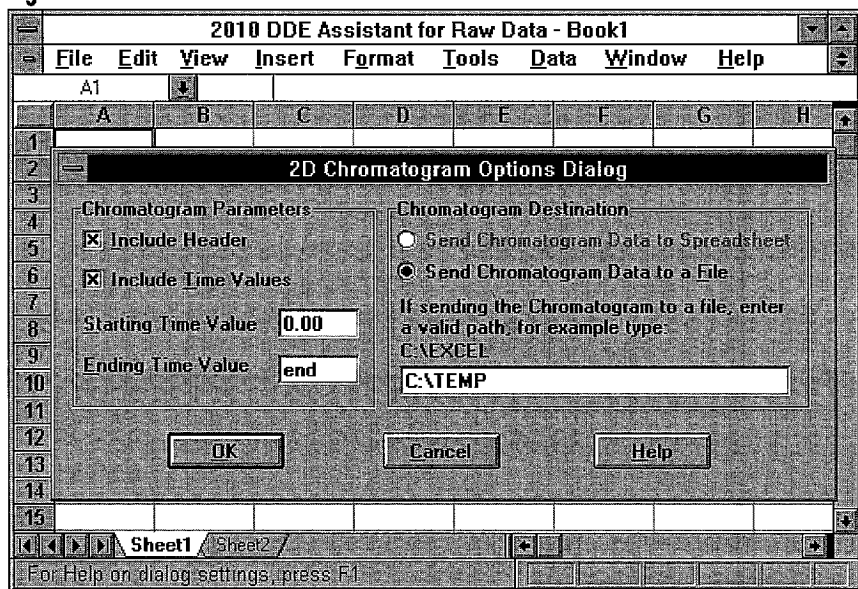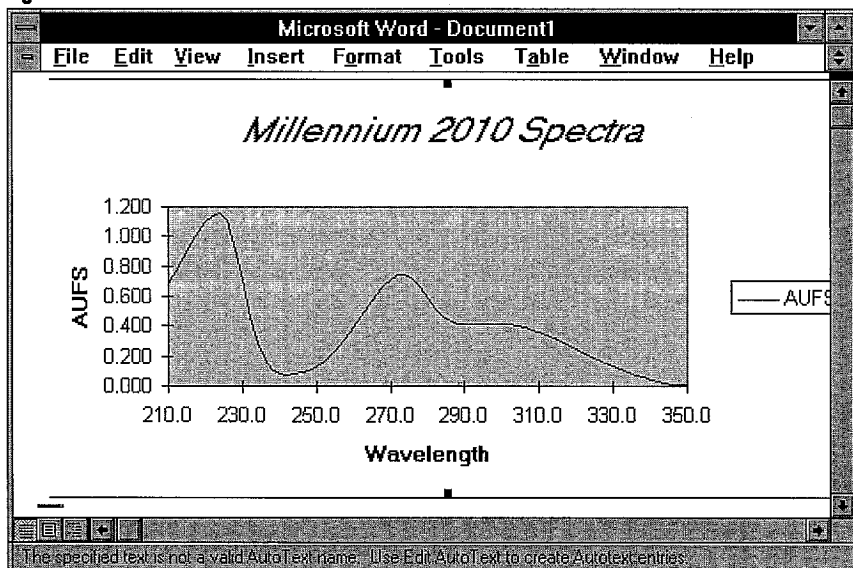
Figure 2

Figure 3

Have You Heard?

The Waters International Network Products User Conference

Waters International Network Products User Conference is a biannual technical conference designed for Millennium and ExpertEase™ users on Network Products, GLP/GALP issues, New Software Applications and Future Strategies. The most recent User Conference was held at Walt Disney's Yacht and Beach Club Resorts, Lake Buena Vista, Florida April 26-29, 1994. The conference covered:

- ExpertEase Networks
- Millennium Client/Server Networks
- Millennium Toolkit
- Future Directions
- Method Validation/System Suitability
- Software Validation
- GLP/GALP Issues

The next conference will be held in Italy, October 11-14, 1994.
If you would like more information, please contact:
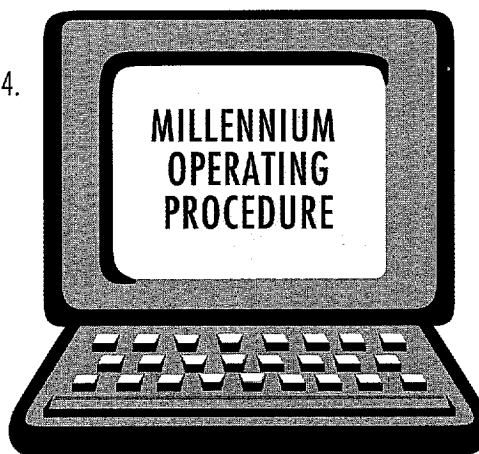Cecile Daly or Dave Levy
Waters
34 Maple Street
Milford, MA 01757 USA
Phone: 508/478-2000 X2555 in U.S.
Fax: 508/478-5591

MILLENNIUM OPERATING PROCEDURE

Waters

Waters Corporation
34 Maple Street
Milford, MA 01757

Patch in to our Network...

with the latest version of the Millennium Chromatography Manager including GPC, System Suitability and networking capabilities.

Please send me information on the following Millennium Products:

3☐ Millennium 2020 C/S Networks
4☐ Millennium Gel Permeation Chromatography
5☐ System Suitability Option
6☐ 996 Photodiode Array Detector
7☐ Integrity System with mass detection

Please add my colleague to the *on-line* mailing list:

Name:

Title:

Company:

Address:

City: State: Zip:

Phone: (   )

PA# 994032