Making Your Own Reference Spectra, Method, and Determining Cell Pathlength Using *AutoQuant 3.x*

**BASIC Training Document**
Midac Applications Department
Peter G. Zemek, Ph.D.

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OVERVIEW

This document is a basic step-by-step instructional guidance manual for users of Midac FTIR instruments using *Autoquant 3.x* version software. By following these instructions, the user will be able to produce high-resolution spectra for the analysis of target compounds of interest. This guide is a reference document used for basic FTIR training. Users may have their own preferences or procedures for completing tasks using FTIR spectroscopy to provide differing results. This document is designed to train the user of Midac equipment and allow them to set-up methods and create spectra for use on Midac equipment using Autoquant software. Midac takes no responsibility for numbers generated by following these procedures as every matrix and method set-up can result in differing results. It is the responsibility of the end user to carefully document how spectra were created, why method routines were chosen, and provide documentation for data quality control (QC) and quality assurance (QA) so that the user can defend his analytical methodology.

If carefully prepared and documented, reference spectra can be successfully applied to obtain accurate analytical results when measuring sample spectra under differing instrumental conditions than those used to measure the reference spectra. However, the best analytical results are usually obtained when reference spectra are measured using the same instrument, temperature, pressure, pathlength, and resolution that will be used to measure the sample spectra for analysis.

Errors can be introduced into the analysis when using spectra produced from outside sources. Examples from these sources of error include the following:

- Erroneous pathlength used when calculating library compound ppm-m
- Non-linearity issues from both hardware, software, or non-linear compound
- Erroneous concentrations determined or manufactured when preparing reference spectra
- Peak broadening occurring from increased temperatures and pressures than those used for analysis
- Peak shifting (x-shifting) occurring from different instruments used to produce the reference spectra or analyze samples
- Appearance of “hot” bands that occur at higher temperatures than those of the reference spectrum
- Baseline anomalies
- Differing window, beamsplitter, or mirror features.
- Contaminants in or incomplete instrument purge when producing background
- Double modulation
- Use of reference spectra apodized differently than the sample spectra
- Etc.
NOTE: If the user is going to make reference spectra that will ONLY be used on their instrument (No transfer of spectra between instruments) then they may skip the procedure for “determining cell pathlength using ethylene” and assume the cell pathlength is arbitrarily “1” or the specified pathlength (0.1, 0.15, 4 or, 10 meters) and proceed directly to “making your own reference spectra.” The pathlength is used to determine pathlength concentration products (ppm-m) that are used to “adjust” the reference spectrum when used on another instrument with a different pathlength. Example: Making a 5 ppm CO₂ reference spectrum, calling the cell 4 meters, assigning the reference spectrum a 20 ppm-m value and entering into the software under the Method Edit Parameters “4” meters. The reported concentration will be the same if the user arbitrarily assigns a “1” to the cell, assigns the reference spectrum 5 ppm-m, and entering “1” under Edit Parameters. But, if you use this reference spectrum on another instrument, then you will receive erroneous results.

The first step in producing high quality, accurate, high-resolution spectra is to prepare the instrument properly. The user must connect the FTIR and computer, heat the cell to the appropriate temperature, turn on instrument power, and allow the instrument to stabilize for a period of two to three hours.

1. IF APPLICABLE, fill up the MCT detector with liquid nitrogen (LN2). If your instrument is equipped with a DTGS, then no further detector preparation is needed. If an InAs (indium arsenide) detector is used, please fill with LN2, or allow Thermo-Electrical (TE) cooling to stabilize for two to three hours.

2. Purge the instrument with dry nitrogen by removing two of the ¼” swage type caps located on three sides of the instrument (I-1000, I-2000). Connect a ¼ inch line (Teflon or Stainless Steel is preferred although others are adequate unless they off-gas) to one of the fittings and run the other to the cell inlet. Allow Nitrogen to flow into the FTIR box at approximately 0.5 lpm. Initially, much higher flow rates may be used to quickly purge the box and cell, but the flow must be turned down once analysis is started due to turbulence issues in the box.

In addition, the light pipes (tubes) connecting the cell and FTIR electronics box on the I-2000 model also has connectors for purging. You may purge with nitrogen in-line with the FTIR box according to the diagrams below but, it is recommended that a separate purge be performed on the light pipes at a lower flow rate.

The headspace from large LN2 Dewars can be used to purge the box, however, it must be noted that this gas does contain small amounts of moisture (1-5 ppm) that will show up in the reference spectra generated.

Please Note: A vacuum on the box may be used to generate ref spectra but unless a near perfect vacuum is achieved, then the reference spectrum will contain very low concentrations of CO₂ that will be subtracted from your sample analysis. This procedure is NOT RECOMMENDED for CO₂ generation of reference spectra.
spectra at low ppm levels. Also, unless the vacuum option is selected for your instrument when ordered, then excessive heat may build up in the FTIR box due to the inability to lose heat by convective heat loss. The heat build-up in the FTIR electronics box will reduce the life of the components and void warranty.

Nitrogen Purge Flow Diagram

3. After the instrument has had sufficient time to purge and stabilize, then the instrument checkout must be performed. Open *Autoquant 3.x* and select the **Setup** pull down tab at the top right side of the page. Select the **Align** button and view the pop-up window.

- Select 40 for points before and after zpd
- Select 32 for resolution
- Select 1 for the gain
- If applicable, depending on your version of AQ, uncheck the box for “compute single beam and/or absorbance
- Select Ok and wait approximately 60 seconds for DTGS to start. MCT will start almost immediately.
The following pop-up window will appear:

![Interferogram Display](image)

The display shows an interferogram (ifg). The important number to review is the max number. The max number should be between 14,000 and 30,000 with a nitrogen purged system. If the max number is between these two limits, then click Exit and **proceed to Step 5**.

If the “Max” number exceeds 32,768 (clipping) or is below 14,000 (low throughput) then you must adjust the detector Gain Setting. Note that the “Min” number may also not be below –32,768. The fixed mirror alignment may also need adjustment but is **not recommended** unless the user is familiar with the procedure.

The next important number is the “ZPD Changes” number and “ZPD Glitch Count”. A few changes are OK, especially when the instrument is warming up or MCT detector is cooling off. If either number starts to count up rapidly, then the system is in need of repair or adjustment. Please call Midac customer support (949)-660-8558 USA.

**GAIN SETTING ADJUSTMENT:**

To adjust the gain setting, perform the following:

Open up the top of the instrument with the 10 hex-key bolts. If you have an MCT, then also remove the 5/8” nut on the detector fill tube and slowly pull the fill tube up and out of the detector. The internal gain adjust board is located on the detector preamp and adjacent to the cylindrical detector (MCT) or card (DTGS).
Adjust the jumper setting to change the max level. Set around 25,000.
You can have multiple jumpers in ON position. By adding more resistors in parallel you will attenuate the detector signal. Jumper A has most attenuation, Jumper H has lowest (highest GAIN). It is recommended that a series of three (3) jumpers be used to obtain a 14,000 to 30,000 Max number.

For more experienced users, a collected interferogram can be transposed to a singlebeam spectra down to 0 cm⁻¹ to observe if the MCT detector is being operated “Too hot”. If an MCT detector is operated in this configuration, it can result in non-linear problems when measuring target species, negative absorbance peaks, or anomalies in the absorbance spectrum. This procedure is performed by first taking a 16-scan interferogram in Autoquant and then opening the interferogram in a spectral manipulation software package such as Grams_32. In Grams, open the interferogram by choosing “file”, “open”, click on the “collect” tab, then “parameter settings”. Set the “spectral range” to go to 4500 to 0 cm⁻¹. Close the window, open “arithmetic”, chose “I-compute” tab, and choose OK. The single beam spectrum will be displayed in the open window. Examine the singlebeam arbitrary y-value around 400 cm⁻¹. The energy with KBr or MCT optics should eliminate all energy (close to zero y value) below this region. If it does not indicate very low energy, then too many photons are hitting the detector. It is then recommended that a screen be used in front of the detector to cut down on the photons striking the detector. (contact Midac applications department for more details 919-522-2032 or 919-577-2215)

**Fixed mirror alignment: (perform only if familiar with procedure)**

The fixed mirror alignment adjusts the rotation of the fixed mirror to optimize throughput. This alignment need not be performed routinely. This procedure should only be performed after rough shipping or damage to the FTIR.
The “Max” number is an arbitrary relative measure of infrared throughput and is related to the number of bits in the ADC. The value can be changed electronically or by physical attenuation. Adjust the hex keys and watch peak height, NOTE: Very small movements result in very dramatic changes in the interferogram, do not exceed 32,768.

4.) After you are satisfied that the FTIR is now functioning properly, click Done in Align Mode. Return to the Collect Tab at top left side of screen.

   Note: You should allow for a minimum of one half-hour hour for the instrument to stabilize after making a fixed mirror adjustment.

5.) At this point, you must know the pathlength of your cell ACCURATELY. In order to do this, you must fill the cell with a known concentration of ethylene gas to determine the pathlength. But first, you must set-up a Method to analyze for ethylene. This procedure is identical for making a method for any other target or group of target compounds.
NOTE: Other CTS gasses may be used (i.e. SF6) to measure pathlength or determine dilutions. EPA recommends using a CTS gas that has its absorbance bands used to determine pathlength in the same wavenumber region used for quantifying a target compound.

**Ethylene Method Set-up Procedure (OR TARGET COMPOUND(S))**

**METHOD SET-UP:**

- Click on **Setup** tab at top left hand corner of screen
- Click “Store Temperature and Pressure”
- Click Setup
- Under Temperature and Pressure settings choose **15 seconds** for “Update every:”
- Click “use default value” for Pressure and Temperature
- Put in 1 atm if running cell under ambient conditions and input your cell temperature (as C). Use (25C) for ambient conditions. Click on “New Method.” Type under “Method Description” Ethylene Pathlength
- Click “Ok”
- Click on Collect tab at top left corner of screen
- Click on “Subdirectory” and name Ethylene Pathlength
- Click on “File name” and Name Pathlength
- Click on “Create”

**Note:** If your instrument is setup to read in Temp and Pressure readings through the RS-232 port, then you do not have to use the defaults and only need to click the “Store Temperature and Pressure” box under the Setup Tab.

- Click **Edit…** under “methods” at middle bottom of screen.
- Click the box for “Enable Temperature and Pressure Adjustments”
- Click “Add Compound” and then type in ethylene
- Input your ethylene reference spectrum temperature. Use 121C if using the Midac Spectrum listed below.

**Note:** You should already have an ethylene reference spectrum for your pathlength determination. If you do not, then use the Midac Reference Spectrum “Ety_H19A ethylene, I2001-V, 121C, 1 atm N2, 207.6 ppm-m Gain=001” available from Midac or the Standards Library. If you prefer the EPA spectra, then the 5CS0124B, 225 ppm-m spectrum is good for a 20 ppm standard with a 10M cell.

**Note:** It is best to use a reference spectrum for ethylene (or any other target compound) that was produced at the same temperature as the sample cell temperature you will be using to analyze samples. Autoquant will adjust for Temperature according to the factor T2/T1 absolute, but will not take into account subtle effects from “peak broadening” or “hot bands” that are variably dependent on the compound, but can add error into your analytical confidence. The Midac Library does contain other spectra at other temperatures for ethylene.
- Click “OK”
- Click on the compound name (ethylene) in the Method Box
- Click “add spectrum”
- Click on “from file” or “from standards library” to choose the ref spectrum location. Double click on the file name and the spectrum will be pulled into Autoquant.
- Click on the file name under the compound name and make sure that the ppm-m field is correct. It should say 207.6 “PPM-M” for the Midac spectrum or 225 if using EPA.

**NOTE:** The EPA library spectra are un-apodized spectra that can be used with any other apodization function. This is why they do not appear as “clean” as some of the Midac spectra. The Midac spectra are all collected with a triangular apodization and all sample spectra should be collected with the same apodization as the reference spectra.

- Click the [view] button
- Click the [Add...] button
- Input 800 and then 945 in the second space and then hit [Add]. Repeat the procedure and then also input 965 and 1135. The window will look somewhat like below but show an ethylene spectrum:

![Ethylene Spectrum](image)

- Hit the [Close] button.

- Click on [Parameters...] at bottom of Methods box and the following screen will pop up:
The parameters shown above should be chosen except for the number of scans. The scans for a DTGS should be input as 4. An MCT should be 8. You can increase the number of scans to improve data quality and increase signal-to-noise. An increase by $2^n$ scans will improve the s/n by a factor of 2 up to approximately 256 scans.

**NOTE:** The resolution must be chosen the same as the reference spectrum

**NOTE:** The pathlength input field should show the pathlength of the extractive cell when analyzing samples. However, when determining the pathlength, we do not know this value. Therefore, you can input the ethylene cylinder value (i.e. 20 for 20 ppm) in this field. When you input this value, the software will report the exact pathlength as the concentration in meters. After the pathlength is determined, then this field must be entered as the actual empirical pathlength value.

20 ppm in N2 balance is recommended for a 10M cell.
40-50 ppm in N2 balance is recommended for a 4M cell.
2000 ppm in N2 balance is recommended for a 10cm cell.
20,000 ppm in N2 balance is recommended for a 1 cm cell.

- Click **Done**
- Click on the **Calibrate** button. This is a very important step. Leave the “linear analysis” and “use global command” boxes **unchecked**.
- Click **Done**. The Method is now set-up for ethylene analysis.

To now start collecting data for ethylene, perform the following steps:

6.) Click **New** under “Continuous Monitoring” tab
   - Choose “standard collection”
• Input “description” (i.e. “ethylene pathlength determination”)
• Enter “subdirectory” field (i.e. ethylene or pathlength)
• Under “memo” enter the following and should be performed for all data collection to ensure that a data trail is made. (i.e. ethylene/N2,20ppm,25C,4scans,1atm,1gain,10M,11000,DTGS, ZnSe,initials,etc.

Note: All of these memo inputs can be abbreviated to fit in the field. The more info, the better. Temp,scans,press,gain is important. This memo MUST BE changes whenever the sampling parameters are changed. This is easily done by stopping the analysis (click stop then close change the memo, and then click resume).

• It is good practice to check the box for interferogram. This is the data in its raw form. Without the interferogram it is difficult to prove that the data has not been manipulated because it cannot be reproduced from scratch. However, this takes up hard drive space. Sub methods results log and residual are not needed and is the users preference to check and save.
• Under number of samples, “-1” means continually collect data
• Check the “Temperature and Pressure Adjusted” box and click on the rectangular box to its right to ensure that the correct Temp and Press default is being used, if applicable
• Click “Ok”
• Input “Scans” to “4” for DTGS detector or “8” for MCT Detector or user preference.

7.) You are now ready to collect a background spectrum for software use
• Click the Collect… box at the bottom of the pop-up screen under “Background”
• Modify “Description:” field as desired
• Enter a large number for the number of scans since we want a clean background single beam spectrum. Typical numbers are 32, 64 or, 128. The DTGS recommended is 16 or 32 if time permits and 32 or 64 for MCT. You can collect as many as 256 or 500 if the user prefers
• Ensure that the resolution is correctly chosen and the same as your Method resolution
• You must now ensure that the system (FTIR box and Cell) have been properly purged with Nitrogen for the proper period of time. When you have properly provided enough time for proper purging of the system, ensure that the purge flow has been cut back to between 0.5 to 1.0 lpm and click the Collect button
• The FTIR will then start to count up the number of scans you selected and then show a “single beam spectrum” .sb. Click “done” when the window pops up but do not hit the primary window “Done” until after you have inspected the single beam
You must inspect the single beam spectrum visually to determine if the quality is good. The spectrum should look somewhat like below:

The single beam ratio is $22/66 = 33\%$
The top single beam spectrum shows a proper energy ratio between the high and low wavenumbers and is indicative of a properly functioning instrument. The bottom single beam shows a properly functioning instrument but also shows a not completely purged system. The wavenumbers at approximately 1400-2000 and 3500-4000 are indicative of moisture (H₂O) molecules in the system. It is very difficult to remove all of the water and some water is OK. The wavenumbers at 2300 to 2400 are CO₂ and are can be purged out of the system. A very small amount of CO₂ is OK if you are not measuring CO₂. When measuring CO₂ it is imperative that it be completely removed or the software will subtract that CO₂ amount from your sample and bias the CO₂ concentration in your sample low.

- If you are not satisfied with the single beam, then hit [Collect] button again. The software will prompt you to overwrite the previous one and click OK.
- When you are satisfied, click [Done] and the software will use the last collected single beam to collect the data unless told otherwise.

8.) You are now ready to determine the Pathlength using ethylene.
   - Disconnect the nitrogen line to the cell only
   - Do not exceed 30 PSI on cell. 10 PSI is adequate on regulator. Flow 2 lpm of Ethylene through the cell for an appropriate amount of time to get at least 5 cell turnovers (0.2 L volume in 4M cell; 2 L Volume in 10M cell)
   - Click the [Start] button at the top right hand side of the screen
   - The Graph colors window will popup. Click OK and the following window will popup and start counting down the number of scans:
The bottom right hand side of the screen will show the absorbance spectrum while the left bottom side will show the pathlength being reported as the concentration. Allow the pathlength number to stabilize and when the graph shows the ethylene pathlength as stable, record the cell pathlength.

The ethylene spectrum should look something like below:
• When you are satisfied, hit the stop button at the top left hand side of the screen
• Now hit the Close button on the right hand side of the screen

9.) You are now ready to make your own reference spectrum

• Reconnect the Nitrogen gas stream to the gas cell. Also keep the FTIR box purging (approximately 0.5-1.0 lpm)
• Click on the Library Tab at the middle of the screen
• Click New Group
• In “New Group Name” type in Field (i.e. CO2)
• In “New Directory name” type in Field (i.e. CO2) (can be same as New Group)
• Click on the new compound name under library folder icon (i.e. CO2)
• Click on New Sample… at top of window
• Enter “File name.” In this field, it is a good idea to also have some way of figuring out what level this spectrum will be. You cannot use any DOS command letter such as (?/.,*\+) A dash (-) is alright. Therefore, If your cell pathlength turns out to be 9.84 meters and you are making a 5 ppm CO2 spectrum, then the ppm-m concentration is (5 * 9.84) or 49.2 ppm-m. So, in the file name you may want to use “CO2-49ppm-m”
• Enter “compound name” (i.e. CO2)
• Enter “Concentration (ppm-m)” (i.e. 49.2)
• Enter Memo info as discussed under ethylene pathlength collect data but also include date collected
• Enter “Number of Scans.” As discussed previously, the number of scans will
determine the signal-to-noise (s/n) and quality of the spectrum. It is up to the
user to determine the data quality objectives of the analysis. Some users will
use 32 for DTGS and 64 for MCT. Others will use 128 or 256.
• Ensure that the Resolution is chosen correctly
• Click interferogram, single beam spectrum, and background single beam
  spectrum. Background interferogram is optional. This will enable anyone
  who comes after you to reproduce the reference spectrum and judge its quality
• Click Parameters and ensure that “Gain” is 1, “Apodization” is triangular, and
  “Resolution” is set correctly. “Wavenumbers” should read 650-4500 for ZnSe
  optics and 400-4600 for KBr optics. Advanced users may set these parameters
to their own specifications
• Click [OK]
• Under “Select Background” at top of window, select [New]
• Type in “Description:” (i.e. BKGD for CO2-49.2ppm-m)
• Type in a “File name” (i.e. BG001). Remember no DOS command characters
• “Number of scans” should be larger than the number of scans chosen for the
  reference spectrum. (i.e. 32 selected for ref spectrum, 64 chosen for BG001)
• Ensure “Resolution” is correct
• Click [Collect] and the FTIR will start to collect a background that will be used
  for the new reference spectrum
• After it has finished, visually inspect the single beam spectrum and decide to
  keep or collect another as in ethylene pathlength collection background
discussed in that section
• Click [Done]
• Now disconnect Nitrogen from cell and start to run your target compound gas
  through the cell at approximately 0.5 to 1.0 lpm. Ensure that enough cell
  turnovers have occurred to have a representative sample in the cell. Make
  sure that you are not pressurizing the cell. A small increase in pressure up to
  0.2 PSI is acceptable
• Once you are sure the cell concentration has reached steady-state, click the
  [Collect] button and the FTIR will start to count up the number of scans
  selected
• When the spectrum pops up on the screen, visually inspect and make a note of
  the maximum absorbance number (i.e. 0.2 abs units located on the y-axis). Either
  overwrite file by clicking [Collect] again or [done]
• Change “file name” and add a “-b” or other identifier to the end of the file
  name to distinguish it from the previous file
• Click Collect and repeat process until the max absorbance value reaches a
  steady-state (very close number to previous file)
• When you are satisfied with the spectrum and result, click [done] or repeat
  process
• Click [Done] to get back to the main screen
• Click “Continuous Monitoring” tab at middle left corner of screen
• You have now collected your own reference spectrum

10.) You may now use your reference spectrum in a Method by editing that method or making a new method.
• Repeat the method process spelled out under the Ethylene Method Setup procedure
• To pull in your own reference spectrum, repeat the exercise for “ethylene method setup” but when you click [Add Spectrum] under the compound name in the “Compound/Spectrum” window, you will choose “add from file…”
• Double click under the “Library” folder on the right side middle of screen window or wherever you chose to put your reference spectrum
• Double click on the reference spectrum .abs file and this will pull the reference spectrum into your method
• Choose regions as previously described under the “ethylene method setup” section. (i.e. CO2 2395-2245)
• The collection of data is also the same as described

You will want to collect new backgrounds as needed throughout your analysis of samples. Other functions of AQ can be found in the instrument manual.