Introduction:

The vibrational energies of molecules are in the range of energy carried by infrared light, according to $E=\hbar \nu$. Infrared (IR) spectroscopy is a technique that measures the amount of infrared light absorbed by a molecule as a function of the frequency of the light. The result is a spectrum that reveals information about the vibrational modes of the molecule.

In Fourier Transform InfraRed (FTIR) spectroscopy, the IR light is not dispersed into individual wavelengths. Rather, an interferometer is used to record the spectrum as a function of the distance a mirror moves. A computer then transforms the spectrum from the "distance" domain into the frequency domain. For several reasons, this results in FTIR being much superior to traditional, dispersive IR, making it the most common IR technique in use today.

Because students spend a considerable amount of time in Organic Chemistry using FTIR to analyze organic molecules, this lab will focus on using the instrument to study the relationships between resolution, signal, and noise in spectroscopy instead. All measurement processes involve the detection of random fluctuations in the signal, which are known as noise. This noise ultimately limits the precision and accuracy of the measurement. That is, no matter how well the instrument is constructed, there will be noise due to two factors imposed by nature: 1) the thermal energy in matter is randomly distributed (following a Boltzmann's distribution), and 2) charge and energy are quantized. The following figures of merit, which are interrelated and influenced by noise, will be studied in this lab:

- **Resolution** is the smallest difference in frequency or wavelength between two absorbance peaks that can be separated by the instrument. All other factors being equal, increased resolution means a better ability to detect absorbances (peaks) that are close together.

- **Signal-to-Noise (S/N)** is defined as the mean divided by the standard deviation (Equation 1). An increased S/N means a better ability to detect absorbances at lower concentration levels.

$$\text{Eqn 1: } \frac{S}{N} = \frac{\bar{x}}{\sigma}$$

Due to the way that data are collected by it, the FTIR spectrometer provides an excellent venue for exploring these figures of merit. In this lab, you will analyze data recorded on an FTIR first to study this relationship. You will then investigate some methods of processing data to enhance the S/N. Two such data processing methods that you will use in this lab are:

- **Ensemble Averaging**, also known as "signal averaging," involves acquiring multiple spectra and averaging them (by summing the individual spectra and dividing by the number of spectra summed, $n$). A signal increases to the first power of the number of spectra ($S \propto n$) but noise (which is random, and thus partially cancels itself out) increases to the square root of the number of spectra averaged ($N \propto n^{1/2}$). Thus the signal increases faster than the noise as multiple spectra are averaged, resulting in an increased signal-to-noise ratio:

$$\text{Eqn 2: } \frac{S}{N} \propto n^{1/2}$$
• **Smoothing** involves using one of several algorithms to digitally remove the noise, while hopefully retaining the important aspects of the signal. This is possible, because in many instrumental methods, the true signal amplitudes (y-axis values) change rather smoothly as a function of the x-axis values, whereas noise occurs as rapid, random changes in amplitude from point to point. In smoothing, the data points of a signal are averaged against its neighbors: points higher than the immediately adjacent points (presumably because of noise) are reduced, and points lower than the adjacent points are increased. This naturally leads to a smoother signal. As long as the true underlying signal is actually smooth, it will not be much distorted by smoothing; the noise, however, will be reduced. Because the noise essentially occurs with high frequency and the signal with low frequency, this type of digital processing is a "low pass filter" (low frequencies are passed through but high frequencies are removed). Averaging can be done in either a weighted or an unweighted fashion. Examples of these types include Savitzki-Golay and Moving Boxcar algorithms, respectively. In the Moving Boxcar algorithm, which you will use in this lab, each point in the signal \( Y_i \) is replaced with the average \( S_i \) of \( m \) adjacent points, where \( m \) is a positive integer called the smooth width. Equation 3 gives the formula for a 3-point smooth \( (m = 3) \):

\[
S_i = \frac{Y_{i-1} + Y_i + Y_{i+1}}{3}
\]

Figure 1 shows data smoothed using a 5- and 9-point Moving Boxcar average.

![Figure 1: Raw data and the results of smoothing with 5- and 9-point Moving Boxcar averages. Figures obtained via http://www.chem.uoa.gr/applets/appletsMOOTH/appl_smooth2.html](http://www.chem.uoa.gr/applets/appletsMOOTH/appl_smooth2.html)
**Equipment:** Computer equipped with a spreadsheet program. USB memory stick.

**Safety Considerations:** There are no specific chemical hazards associated with this lab.

**Data:**

Your Instructor has already collected the data listed in Table 1 below via the following procedure:

1. The FTIR instrument was set up using the parameters (Resolution, Scan width, Number of scans 'n') listed in Table 1.
2. A background scan was collected with nothing in the beam of the instrument.
3. A sample scan was collected with nothing in the beam of the instrument.
4. The data were exported to a text file with wavenumber and % transmittance values given in a tab-separated list.
5. Steps 3 and 4 were repeated as needed to obtain multiple data sets.

<table>
<thead>
<tr>
<th>Table 1: Description of FTIR Data Collected</th>
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<tbody>
<tr>
<td>Resolution (cm⁻¹)</td>
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<td>-------------------</td>
</tr>
<tr>
<td>0.5</td>
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<tr>
<td>0.5</td>
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<td>1.0</td>
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<td>2.0</td>
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<td>4.0</td>
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<td>8.0</td>
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<td>16.0</td>
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<td>32.0</td>
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<td>4.0</td>
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</tbody>
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These data have been collected in a .zip file, available on the course website.
Procedure and Analysis:

This Procedure includes the Analysis as well. Not every "Analysis" step needs to be done in your notebook. For example, you will make a number of graphs, but only 6 will be printed and included in your notebook. Information to be included in your Notebook is indicated with an asterisk.

**SAVE YOUR WORK FREQUENTLY!!!**

Getting started

1. Obtain and login to a computer equipped with Microsoft Excel, OpenOffice Calc, or other spreadsheet program.
2. Download and unzip the file "6-FTIR-ResolutionSignalNoise-Data.zip" from the course website. Note that this will create a directory structure where the data are organized by the resolution and experiment type.
3. For each data set, you will need to import the data into your spreadsheet (do not do this all yet!). Familiarize yourself with the format of the data by opening the file 0.5-Resolution\InstAnaly-FTIRLab-05Res-2200-2000.asc in WordPad or other text editor. You should see the data start around line 57. The numbers in the first column are wavenumbers (cm\(^{-1}\)), and those in the second column are % transmittance.
4. Before you begin importing the data in earnest, familiarize yourself with the process of importing data from a text tile into your spreadsheet. Things to think about that may make the process go a little faster: Is there a way to have Excel ignore all the lines before the data start? How can you ensure the two columns of numbers appear in two separate columns of cells? Should new data be imported into the same sheet or a new one?
5. You will be calculating the signal-to-noise ratio (S/N) many times through the course of this lab. The procedure to do this is:
   a) Use built-in functions to calculate the average (\( \bar{x} \)) and standard deviation (\( \sigma \)) of the %Transmittance. **Note:** the average should be 100%, as the "sample" was taken with nothing in the beam! It will probably be very close to, but not exactly 100%.
   b) Calculate the S/N by diving the average by the standard deviation.

Smoothing an initial data set

1. Import the single data set with Resolution = 0.5 cm\(^{-1}\) and Scan width = 2200-2000 cm\(^{-1}\).
2. Graph the data (%Transmittance vs wavenumber). **Note:** the x-axis should have larger wavenumbers on the left.
3. Calculate the S/N of the data set. *Record this value in your notebook.
4. Perform Moving Boxcar smooth of the data with a smooth width of 5 points. *An example of how to do this is given in Figure 2.*

![Figure 2: Initial formula for a 5-point Moving Boxcar smooth](image-url)
5. Graph the smoothed data on a separate graph. *Hint: cutting and pasting the initial graph, then changing the data set may be faster than formatting a completely new one.


7. Repeat steps 4-6 for 9, 15, and 25 points. *The smoothed data should appear in separate columns.

8. Make a plot of S/N versus smooth width \(m\). *Include this in your Notebook. *Comment on the shape of this plot.

**Resolution versus Signal-to-Noise**

1. Import the 4 data sets with Resolution = 0.5 cm\(^{-1}\) and scan width = 3000-400 cm\(^{-1}\). Place all of this data in one spreadsheet in four neighboring columns.

2. Perform an Ensemble Average in a fifth column by averaging all four %transmittance values at every wavenumber.

3. Calculate the S/N of the averaged data in three regions: 3000-2800 cm\(^{-1}\); 2200-2000 cm\(^{-1}\); and 600-400 cm\(^{-1}\). *Record this value in your notebook.

4. Make two graphs of %Transmittance vs wavenumber: 1) using one of the original data sets, and 2) using the Ensemble Averaged data.

5. Repeat steps 1-3 (but not 4!) for the data collected at resolutions 1.0, 2.0, 4.0, 16.0, 32.0 cm\(^{-1}\). *Note: each resolution should have 4 data sets to be Ensemble Averaged.

6. Make three plots of S/N versus Resolution (one for each of the three wavelength regions in which you calculated S/N). *Comment on the shape of these plots. *Include these in your Notebook.

7. Import the background data for Scan width = 3000-400 cm\(^{-1}\). Graph this and use it to discuss why the S/N varies in the three regions of wavenumbers.

**Ensemble Averaging**

1. Import the six data sets with Resolution = 4 cm\(^{-1}\), Scan width = 2200-2000 cm\(^{-1}\), and number of scans = 1, 4, 16, 64, 256, and 512. Place all of this data in one spreadsheet in six neighboring columns. *Note: these are all in the same directory 4.0Res-EnsAvg.

2. Calculate the S/N of each data set. *Record this value in your notebook.

3. Plot S/N versus number of scans. *This should not be a straight line!

4. Plot ln(S/N) versus ln(number of scans). *Find the slope of this line. *Comment on how close the slope is to 0.5 and why this would be. *Include this graph in your notebook.

**Resolution and Signal-to-Noise in a real spectrum**

1. Go to the FTIR instrument and collect your own spectrum of CO\(_2\) in the following way:
   a) In the "Setup" menu, choose "Instrument." *A couple pop-up windows with errors will appear. Just hit "OK."
   b) Under the "Samples" tab, enter a useful filename in the appropriate box
c) Under the "Scan" tab, enter the following parameters:
   • Start = 2400 cm⁻¹
   • Stop = 2200 cm⁻¹
   • Scan type: background (IMPORTANT!)
   • Units: %T
   • Scans: 16
   • Resolution: 0.5 cm⁻¹

d) Hit the "Apply" then "Scan" buttons. Note: at this resolution, scans take a few minutes. The machine may appear to be doing nothing, but it is! Be patient and do not hit any more buttons.

e) When the instrument has completed the scans, the spectrum will pop up. This is not an absorbance or transmittance spectrum, because it was recorded as a background. In other words, the background has not been subtracted out, and you are essentially looking at I₀. You should see a series vibrational/rotational transitions for gas phase CO₂ in the atmosphere.

f) Export the spectrum to a text file using "Save As" and selecting the type "ASCII" with the suffix .asc. Save the .asc file to a USB memory stick.

g) Collect two more spectra of CO₂ at resolutions of 8 and 32 cm⁻¹

2. Return to the lab and import your spectra into your spreadsheet. Note: you will NOT calculate the S/N for these spectra.

3. Graph each data set on a separate graph.

4. Using the 0.5 cm⁻¹ resolution data, repeat the same Moving Boxcar smoothing routine you did previously with 5, 9, 15, and 25 points.

5. Graph each smoothed data set on a separate graph.

6. *Comment on the effects of changing resolution and smoothing on this "real" spectral data.

Lab Report including Conclusions and Discussion

Your lab report is due by lecture on Wednesday, . Your report must be handed in BOTH electronically (via Moodle) and in hard copy form. See the document "LabReportGuide.pdf" on the course website (http://oz.plymouth.edu/~jsduncan/courses/2011_Fall/InstrumentalAnalysis) for guidelines on writing your report.

In addition to the Analysis performed in your notebook, include the following in your report (place them in the most relevant sections):

1. Include the 6 graphs you were instructed to include in your Notebook.
2. Expand on the "comments" you included in your Notebook. Include additional figures that help illustrate your comments.
3. Discuss the relationship between resolution and S/N.
4. Discuss the uses of ensemble averaging and smoothing. What are the advantages and disadvantages? What, if any, are the limitations to their usage?