1.0 Overview of the Course

- why study chemical instrumentation?
- Purdue's historical and continuing contribution
- instrumentation "ignorance" leads to bad data
- key design considerations
- assessing analytical sensitivity
- evaluating selectivity with information theory
- contents of CHM 424
Why Study Instrumentation?

• Sir Humphry Davy said, "Nothing begets good science like the development of a good instrument."
• the core activity of science involves testing hypotheses through measurements, and measurements are usually made with instruments
• refined instrumentation improves the quality of data permitting a wider range of samples, e.g. tandem mass spectrometry
• new instruments open up whole new fields of investigation, e.g. scanning tunneling microscopy and lasers
• there will always be a need for scientists with the specialized knowledge required to design and construct instrumentation
• anyone using chemical instrumentation will benefit by knowing the physical and electronic principles of operation
ACS Award in Chemical Instrumentation

- **1971:** Fred McLafferty, was a professor at Purdue from 1961-68, mass spectrometry instrumentation
- **1979:** John Walters, was an undergraduate at Purdue in the 60s, atomic emission instrumentation
- **1981:** Jonathan Amy, created the chemistry department instrumentation facility, designed and built the first recording infrared instrument and contributed gas chromatograph design
- **1982:** Harry Pardue, designed the first array detector using a television vidicon tube, instrumentation for clinical analyses
- **1984:** R. Graham Cooks, developed the area of tandem mass spectrometry, Fourier Transform ion traps, and multiplexed mass spectrometry detectors
- **1986:** Fred Lytle, developed the synchronously-pumped dye laser, fast photon counting strategies, two-photon excitation in small volumes
- **1991:** Joel Harris, Purdue graduate student in the early 70s, developed photo-thermal detection methods
- **2007:** Scott McLuckey, mass spectrometry instrumentation, gaseous macro-ion chemistry, proteomics
Major Instruments Developed at Purdue

- scanning prism-grating infrared spectrometer
- preparative gas chromatograph
- high resolution mass spectrometer
- high resolution gas chromatography
- laboratory computer control of instrumentation
- mass-analyzed ion kinetic energy spectrometer
- NMR instrumentation
- diode array spectrometers
- tandem mass spectrometry
- ion trap mass spectrometry
- picosecond dye laser
- fast photon counting instrumentation
- asynchronous optical sampling
- robotics
- Fourier transform ion cyclotron resonance
- pulsed-accelerated flow kinetic spectrometer
- Raman imaging spectrometer
- chip-based separations and reactions
- ion trap arrays
Some Current Instrumentation

• **McLuckey**: linear ion trap mass spectrometers; quadrupole and triple quadrupole mass spectrometers designed for ion/ion reactions
• **Shepson**: designing instrumentation for airborne measurements in both airplanes and blimps; designing remote instrumentation placed at the top of towers
• **Kissinger** (BAS): automated instruments and associated software for obtaining representative samples of physiological fluids; massively parallel measurements on these samples
• **Kenttämaa**: laser-driven acoustic desorption of macromolecules
• **Raftery**: multiplexed NMR measurements
• **Cooks**: an array of $10^6$, 1 μm ion trap mass spectrometers; high throughput mass spectrometry using four independent sources, analyzers and detectors; a small fieldable (25 lbs) mass spectrometer; new type of high performance spectrometer based on orbital ion motion; an instrument for preparing mass-analyzed arrays of protein spots on a chip
• **Simpson**: laser-based instruments for non-linear optics at biological interfaces
It is rather common for people to say the dumbest things because they do not understand the physical principles behind a measurement nor how the instrument works. It is truly embarrassing when these statements get published, or worse, entire studies invalidated.

- not realizing that most instruments need to be calibrated (absorption spectrometer 150% in error)
- not thinking about the how the measurement is made (reporting an absorption of 5, which would require that the spectrometer have virtually no stray light)
- not knowing how the numeric values in the computer were obtained (analog-to-digital converters often have a poor accuracy and can change the frequencies of a measurement)
- not knowing that a high signal-to-noise ratio was probably obtained by using an electronic filter (they distort peak shapes and positions)
- proposing an experiment that voids established theory (high resolution picosecond spectroscopy and the Uncertainty Principle)
Key Design Considerations

- quantitation and qualitation have different measurement needs (single measurement at a high signal-to-noise ratio compared to a feature-rich measurement)
- single-application instruments are often simpler than general purpose instruments (filter fluorimeter compared to a spectrofluorimeter)
- complex samples require either a separation step to simplify the composition and/or a high resolution measurement to distinguish among components (as an example, GC/MS)
- trace analysis requires instruments rugged toward contamination (an instrument that doesn't contact the sample or is easily cleaned between samples)
- sample throughput influences the time scale of the measurement (fast signals have more noise than slow signals)
- automated and manual instruments have different design constraints (reagent addition, sample manipulation, etc.)
Assessing Analytical Sensitivity

• selectivity is the ability to distinguish among sample components
• the detection limit is that concentration yielding a signal equal to three times the noise
• sensitivity is the slope of the calibration curve
• sensitivity can be attributed to three components of a measurement
  ▪ molecular sensitivity (molar absorptivity, fluorescence quantum yield)
  ▪ instrumental sensitivity (spectrometer resolution, fluorimeter source intensity)
  ▪ method sensitivity (extraction efficiency, reaction efficiency, sample size)
• theory can be used to help increase the sensitivity of a measurement by identifying the parameters that can be optimized

\[ I_f(\lambda_e, \lambda_f) = I_s(\Delta \lambda_e) E(\lambda_e)(1-T(\lambda_e)) \Phi_f F(\Delta \lambda_f) E(\lambda_f) \]

• sensitivity and selectivity are always traded against one another
• an increase in sensitivity does not necessarily improve the detection limit
• analytical chemistry is the discipline concerned with testing hypotheses utilizing knowledge of the composition of matter - this knowledge might be of a qualitative, quantitative or structural nature
• analytical procedures are the chemical and instrumental measurements used to obtain data concerning the composition of matter
• the amount of information produced by a given procedure can be determined by the decrease in uncertainty concerning the composition of matter, as long as that decrease serves to test the stated hypothesis
• the definition uses the phrase, "decrease in uncertainty," because we must have some prior knowledge about the sample or assumptions which we are willing to make about the sample
• analytical selectivity is proportional to the information content of an analytical procedure - the higher the information content the easier it is to select among various possible sample identities
Information Theory

• the basic unit of information is the bit - it corresponds roughly to choosing yes or no in a 50:50 situation
• zero bits would correspond to observing a totally expected event, while infinite bits would correspond to observing a totally unexpected event
• less than one bit of information can be conveyed
• Shannon's formula relates probability to information content in bits
  \[ I = \log_2 \left( \frac{1}{P} \right) \]
• in analytical chemistry we define the information content of a measurement by how much it reduces the uncertainty of the composition of matter
  \[ I_{\text{gain}} = U_{\text{before}} - U_{\text{after}} \]
  • \( I_{\text{gain}} \) depends upon the measurement, while \( U_{\text{before}} \) depends upon the sample and the hypothesis
Consider instruments such as burets, balances, flame photometers, chromatographic retention times, etc. These devices have a range of readings. If all possible readings have the same probability before the measurement is made, then the maximum information that can be gained is given by the following.

\[ I_{\text{gain}} = U_{\text{before}} - U_{\text{after}} = \log_2 \left( \text{range} \right) - \log_2 \left( \text{resolution} \right) = \log_2 \left( \frac{\text{range}}{\text{resolution}} \right) = \log_2 S \]

where \( S \) is the number of steps in the measurement.

In some cases it might be more appropriate to postulate that the probability of making any measurement is normally distributed, and that the noise is also normally distributed. For this case the maximum information that can be gained is given by a similar expression.

\[ I_{\text{gain}} = 0.5 \log_2 \left( \frac{\sigma_{\text{before}}^2}{\sigma_{\text{noise}}^2} \right) \]
Information from Multiple Measurements

- The information from uncorrelated measurements will add.
  - refractive index: 1.3 - 1.7 measured to 0.0001 = 12 bits
  - boiling point: 50 - 250° measured to 0.1 = 11 bits
  - both: 23 bits
- Repeating the same measurement will increase information by one bit for each factor of four increase in the data.
- Using multiple uncorrelated measurements gains information faster than improving precision.
- Spectra can be thought of as multiple measurements, thus a spectrum could provide a huge amount of information,

\[ I = S_x \log_2 (S_z) \]

where \( S_x \) is the number of steps on the x-axis (number of measurements) and \( S_z \) is the number of steps on the z-axis.
- For higher dimensional data, e.g. 2D NMR or GC/MS, the above equation is multiplied by \( S_y \). This is why multidimensional data has such great selectivity.
Weakness of Simple Information Theory

- Some data have a highly probable z-axis value, but $\log_2(S_z)$ is based on $P = 1/S_z$. Examples are atomic emission and Raman, both which have sparse spectra (high probability of $z = 0$).
- Some data are highly correlated. As an example, for data that decay exponentially the information does not linearly increase with temporal resolution.
- Some data are moderately correlated. For example, the band shapes of absorption spectra are mathematically smooth. Knowing the absorption at one wavelength narrows the possible range of absorption values at adjacent wavelengths.
- Some groups of data are correlated. For example, a fluorescence spectrum is often the mirror image of the absorption spectrum. Thus it does not add new information to the extent predicted by the simple equation.
Course Outline (1)

- analog electronics
  - direct current, resistors, and Ohm's law
  - alternating current, capacitors, and complex impedance
  - operational amplifier circuits
- digital electronics
  - binary representation of numbers
  - digital logic and digital input/output
  - digital-to-analog and analog-to-digital conversion
- optical components
  - refractive index, lenses and mirrors
  - sources, monochromators and detectors
- molecular spectroscopy of solutions
  - quantitative absorption
  - absorption spectra
  - fluorescence spectra
  - quantitative fluorescence
Course Outline (2)

- vibrational spectroscopy
  - molecular vibrations
  - infrared spectroscopy
  - Fourier transform infrared
  - Raman spectroscopy
- mass spectrometry
  - fundamentals of ionization and dissociation
  - ion sources
  - mass analyzers
  - ion detectors