ABSTRACT: 141

NONDESTRUCTIVE COMPOSITIONAL ANALYSIS OF SOYBEAN SEED BY TRANSMISSION RAMAN SPECTROSCOPY: A NEXT GENERATION TECHNOLOGY

KULL L4, SCHULMERICH M1,2, AZAM H1, GELBER M2, HARRISON S3, OWEN B4, MCKINNEY J3, THOMPSON D3, AND BHARGAVA R1, 2

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Reliable, inexpensive, rapid and nondestructive compositional analysis of soybean seeds is important to soybean producers, developers, grain elevators, co-ops, processors, and distributors. Animal diet formulation and plant breeding require reliable information of amino acids, fatty acids and sugar content. Precise and rapid compositional analysis of economically important components has proved to be challenging and is an ongoing area of research. Transmission Raman spectroscopy (TRS) can provide quantitative analysis of soybean chemistry in which the level of chemical specificity, degree of precision, and chemical contrast may offer improvements over currently utilized methods such as near infrared spectroscopy. To investigate the feasibility of using TRS for non-destructive soybean analysis, we built two soybean Raman instruments, one for measurements on single soybeans and one for bulk sample analysis.

Utilizing the single soybean instrument, we collected Raman spectra from over 1000 soybeans. Subsequent wet chemistry was performed to evaluate the actual protein and oil content. Collected Raman data were processed with a standard partial least square (PLS) calibration approach. The root-mean-standard error of prediction (RMSEP) for the oil calibration was 0.89%. Prediction capabilities were similar for protein content with a RMSEP of 0.92%. In both calibration and validation sets, the predictive capabilities of the model were similar to the error in the reference wet chemistry method. We then extended our methods to bulk analysis of 52 different soybean samples analyzed for 23 amino acids and five fatty acids. Of the 23 amino acids, 13 had an R^2 value >0.7, and all five fatty acids had R^2 values >0.7. This study is the first evidence of nondestructive TRS compositional analysis of soybean seeds both in single and bulk samples. Our results indicate that Raman spectroscopy can be used effectively in quantifying specific soybean seed components. Work towards improving and expanding the calibration models is currently underway in our laboratories. The latest results will be presented.

Beckman Institute
at The University of Illinois

National Soybean Research Laboratory

Compositional analysis of soybean seed by Transmission Raman spectroscopy

Matthew Schulmerich, Michael Walsh, Matthew Kole, Matthew Gelber, Rong Kong, Sandy Harrison, John McKinney, Dennis Thompson, Linda Kull, Bridget Owen, and Rohit Bhargava

Acknowledgments

- Matthew Schulmerich
- Michael Walsh
- Matthew Kole
- Matthew Gelber
- Rong Kong
- Sandy Harrison
- John McKinney
- Linda Kull
- Rohit Bhargava

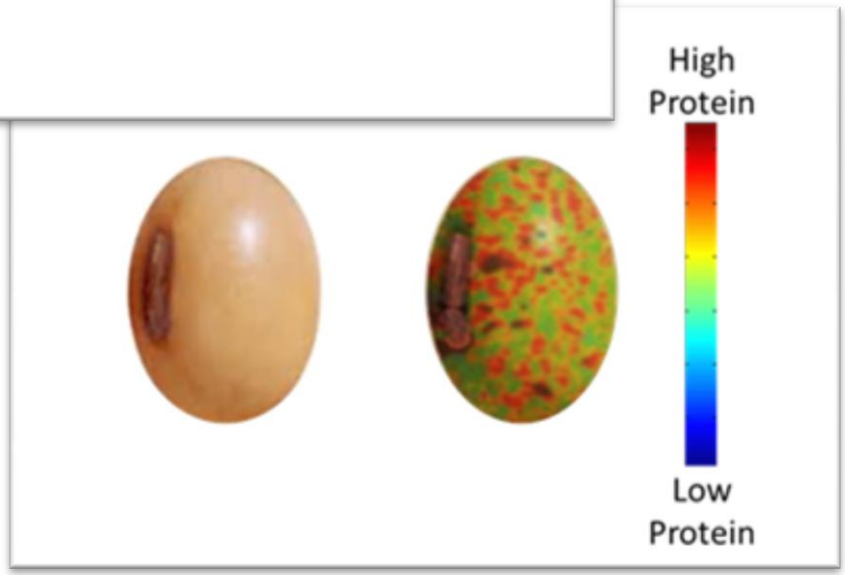


Thank you



Soybeans

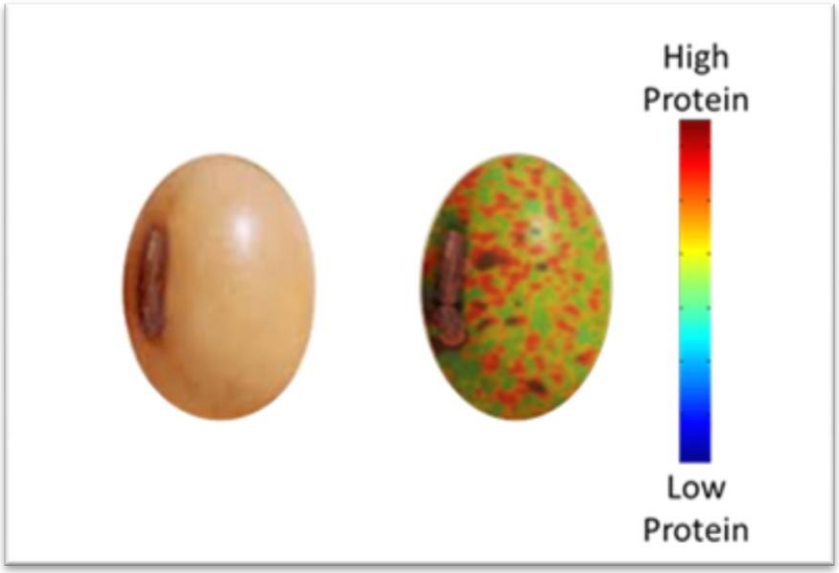
Not all soybeans are created equal... premiums are paid for nutritional content!



- 2010 soybeans were grown on over **76 million acres** of land in the United States
- This produces 90 million metric tons of soybeans worth **~$40 billion**
- US Soybean and product exports exceeded **$23 billion**

*** American Soybean Association (Soy Stats 2011)

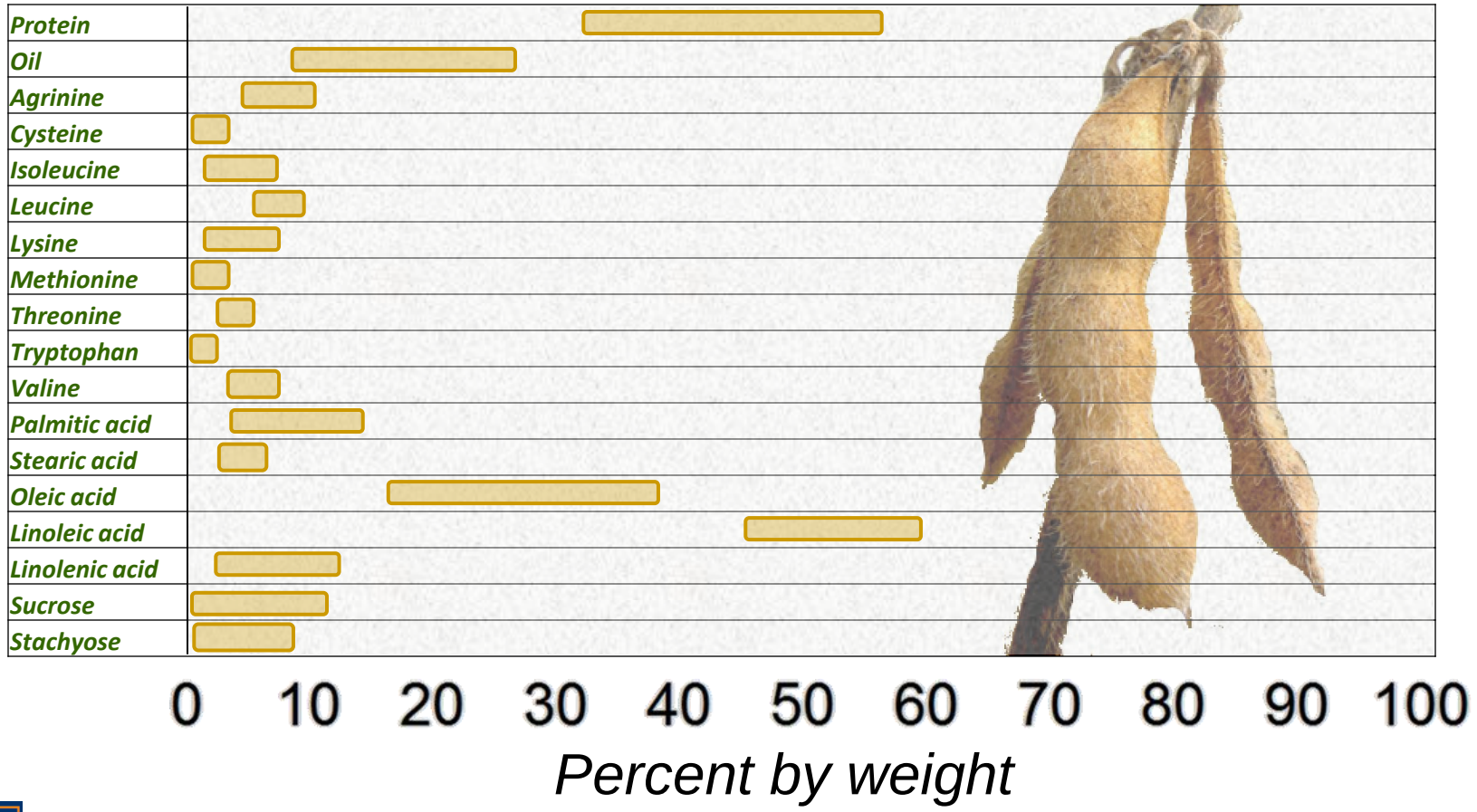
Soybeans



- Soybeans Components Have the Value
  - Protein
  - Oil
  - Amino Acids
  - Fatty Acids

Nutritional Content

Buyers are interested in quantifying:



*** Germplasm Resources Information Network - (GRIN)

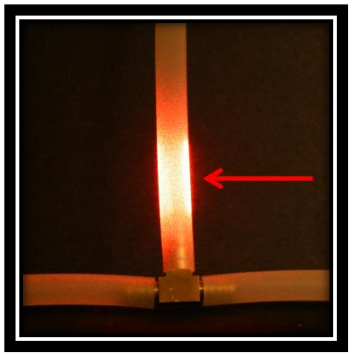
Project Background

University of Illinois Raman Spectroscopy Research for Medical and Pharmaceutical Applications

Exploration of this Technology for Agricultural Applications

The General Problem

The general problem is light scattering:



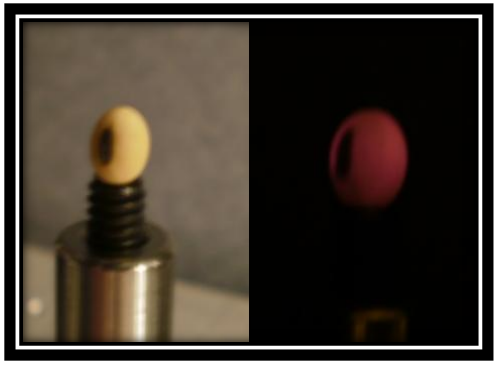
Process Analytical Technology



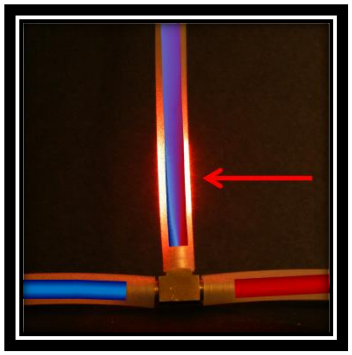
Biomedical Applications



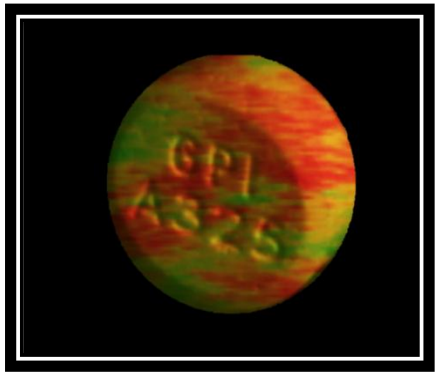
Pharmaceuticals

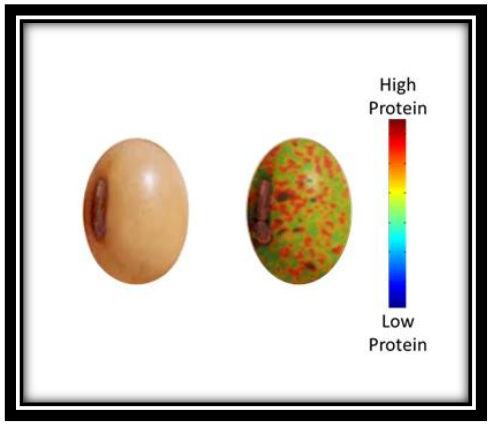


Agriculture





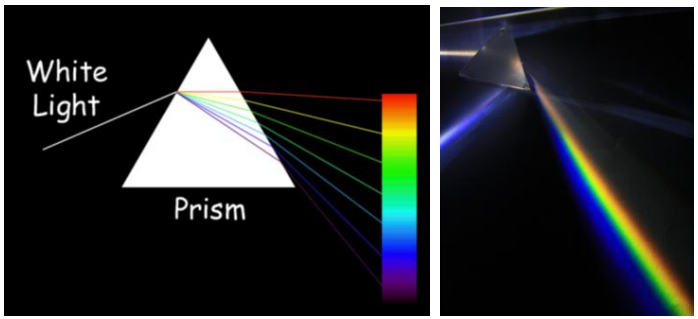




What is Spectroscopy?

A Rainbow is a visible spectrum





Spectroscopy is converting light into information:





What is Spectroscopy?

The Human Eye only Sees 3 colors

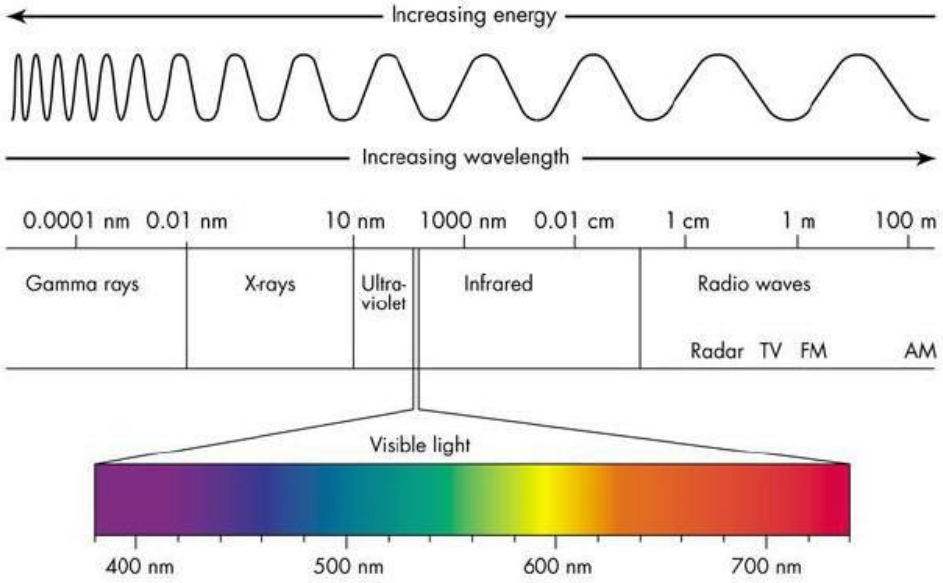




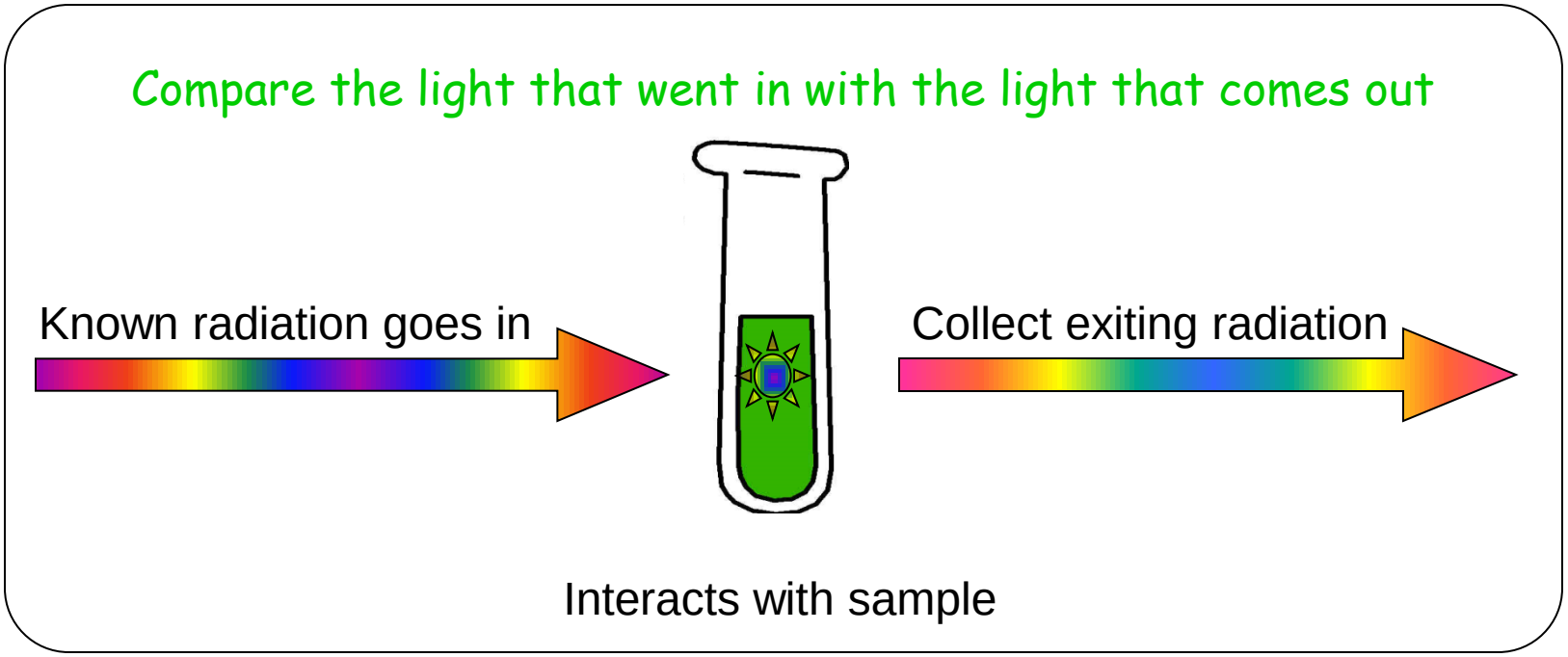
Spectroscopy is converting light into information:

-Can be qualitative, ie. Observing what colors are present or not present

-Can be quantitative ie. Observing the intensities of each color that is present.

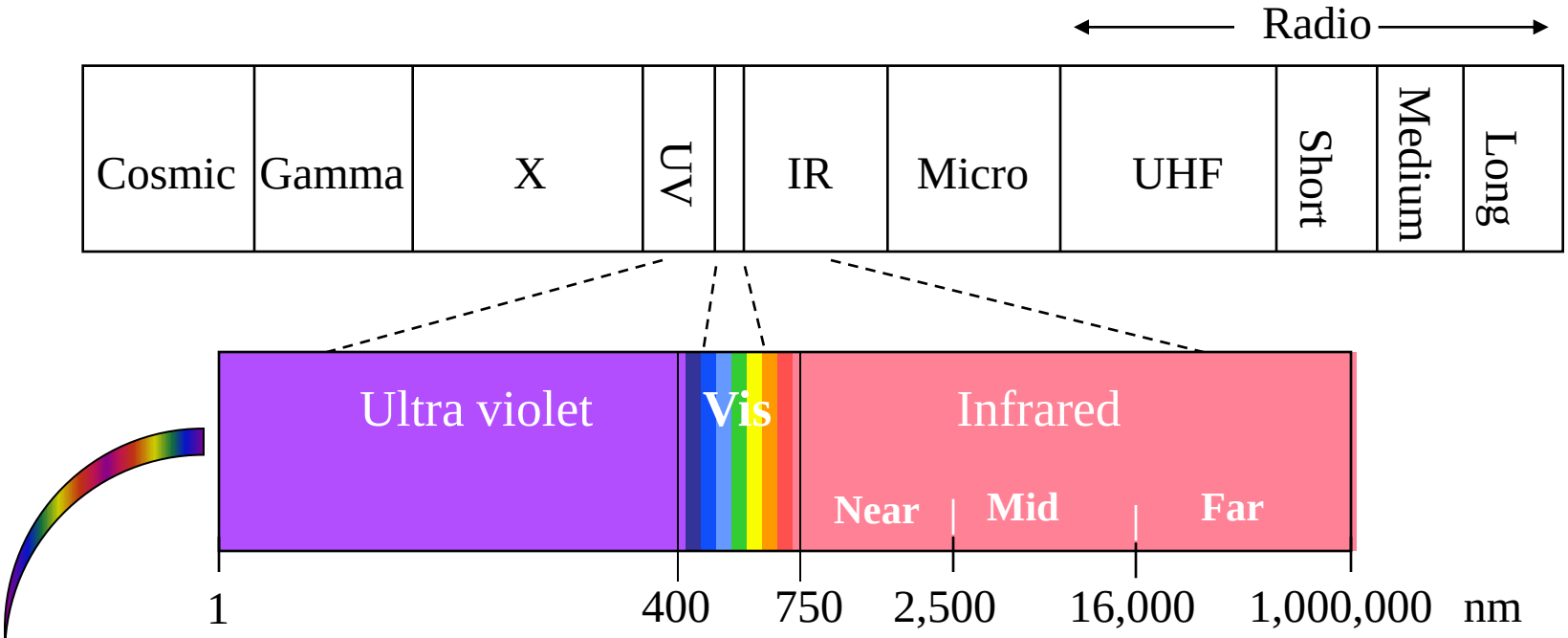


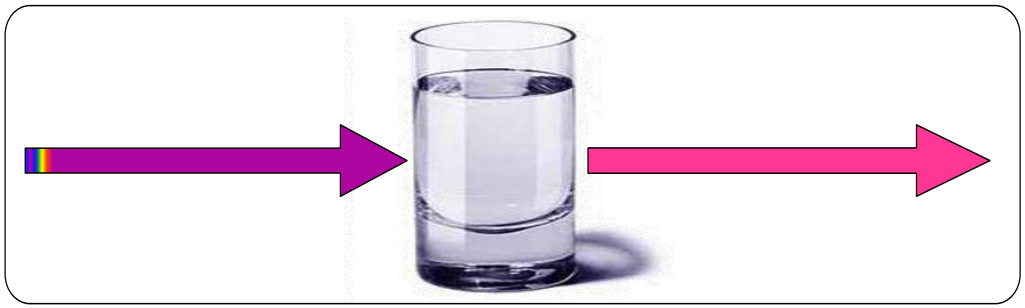
Spectroscopy generalization



A general definition of spectroscopy is the study of the interaction between radiation and an analyte as a function of wavelength.

Spectroscopy (Radiation)





To see a spectrum we plot absorbance vs. wavelength



Spectroscopy Generalization

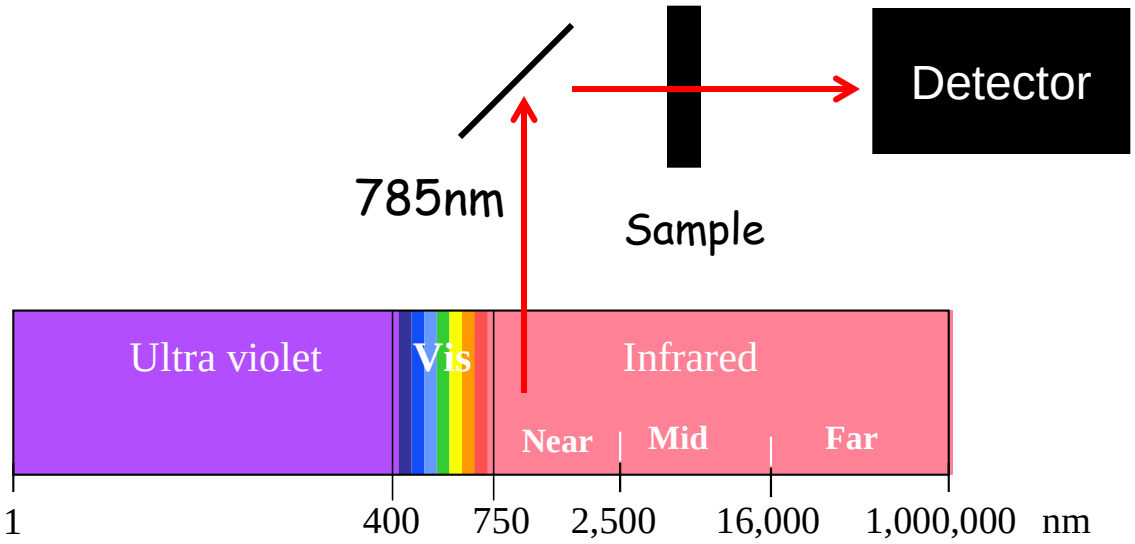
Reasons for Light absorption:

UV and visible spectroscopy: ‘Electronic spectroscopy’, light absorption occurs as a result of electrons in molecules moving to higher energy orbitals

Infrared and Raman Spectroscopy: ‘Vibrational spectroscopy’, spectral bands occur as a result of molecular vibrations



Raman Spectroscopy: Basic Concept

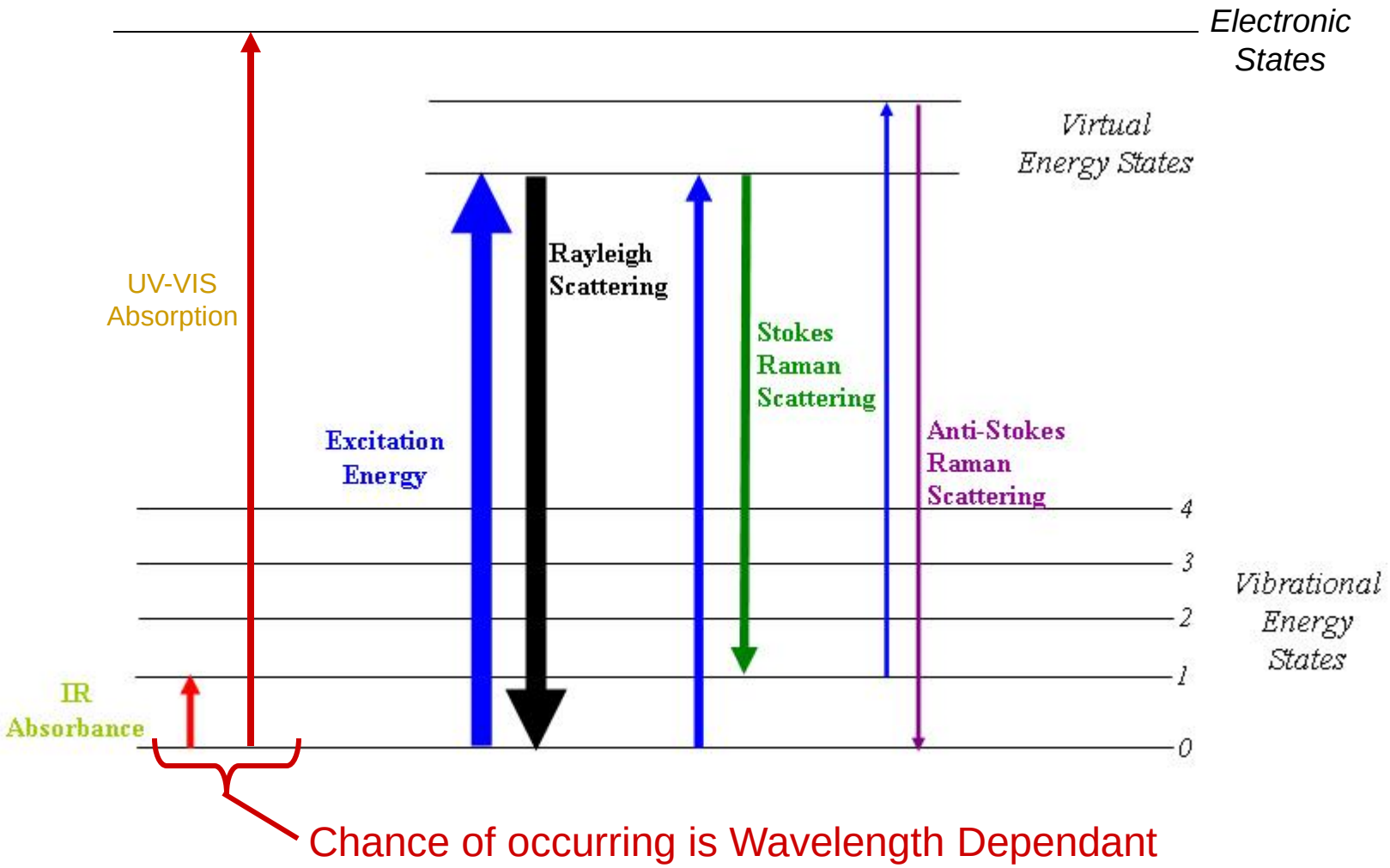




First observed by Sir C. Venkata Raman in 1928 using sunlight and photographic filters (won Nobel price in physics in 1930).

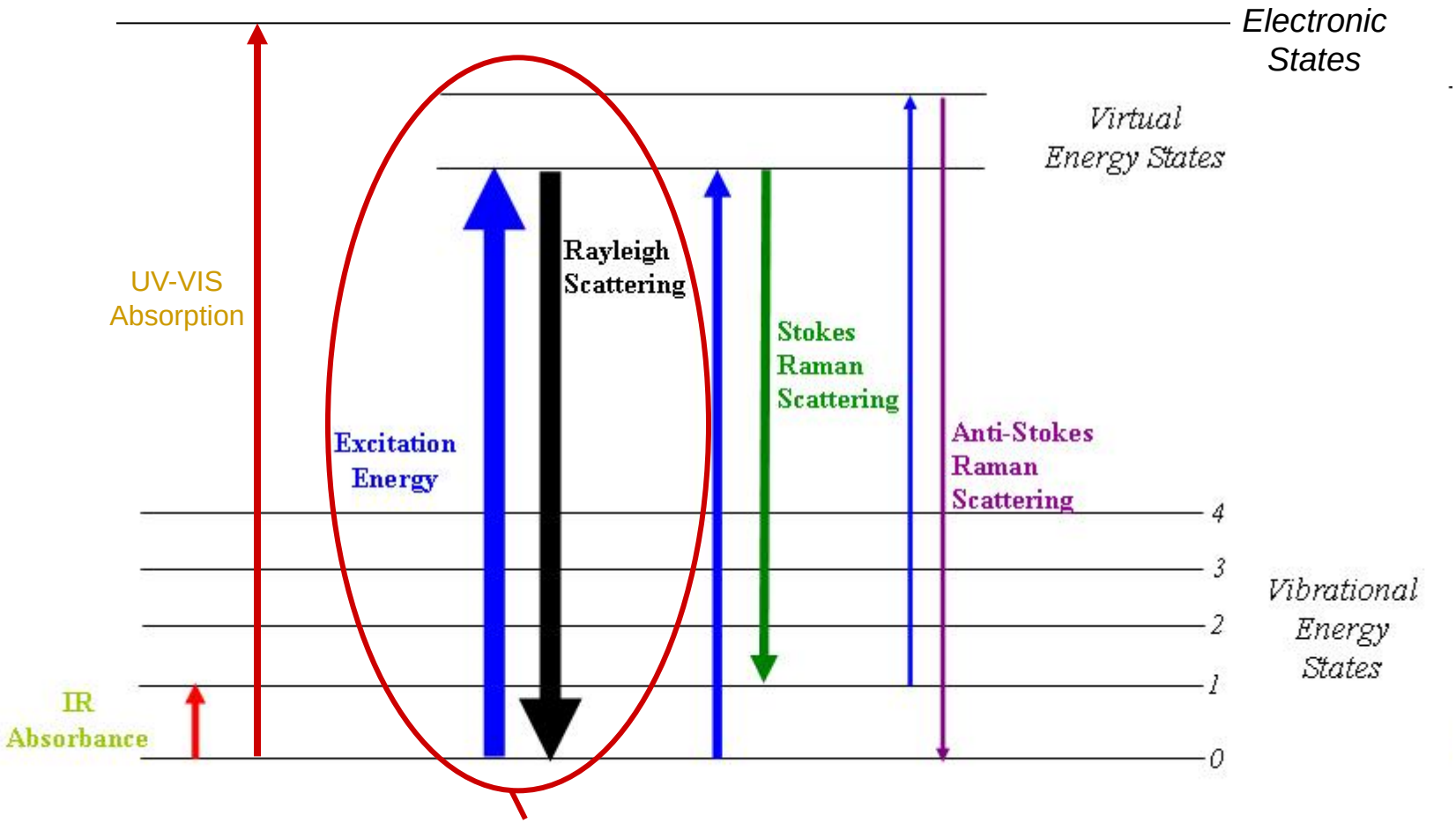
Nobelprize.org

Raman Spectroscopy: Basic Concept



http://en.wikipedia.org/wiki/File:Raman_energy_levels.jpg

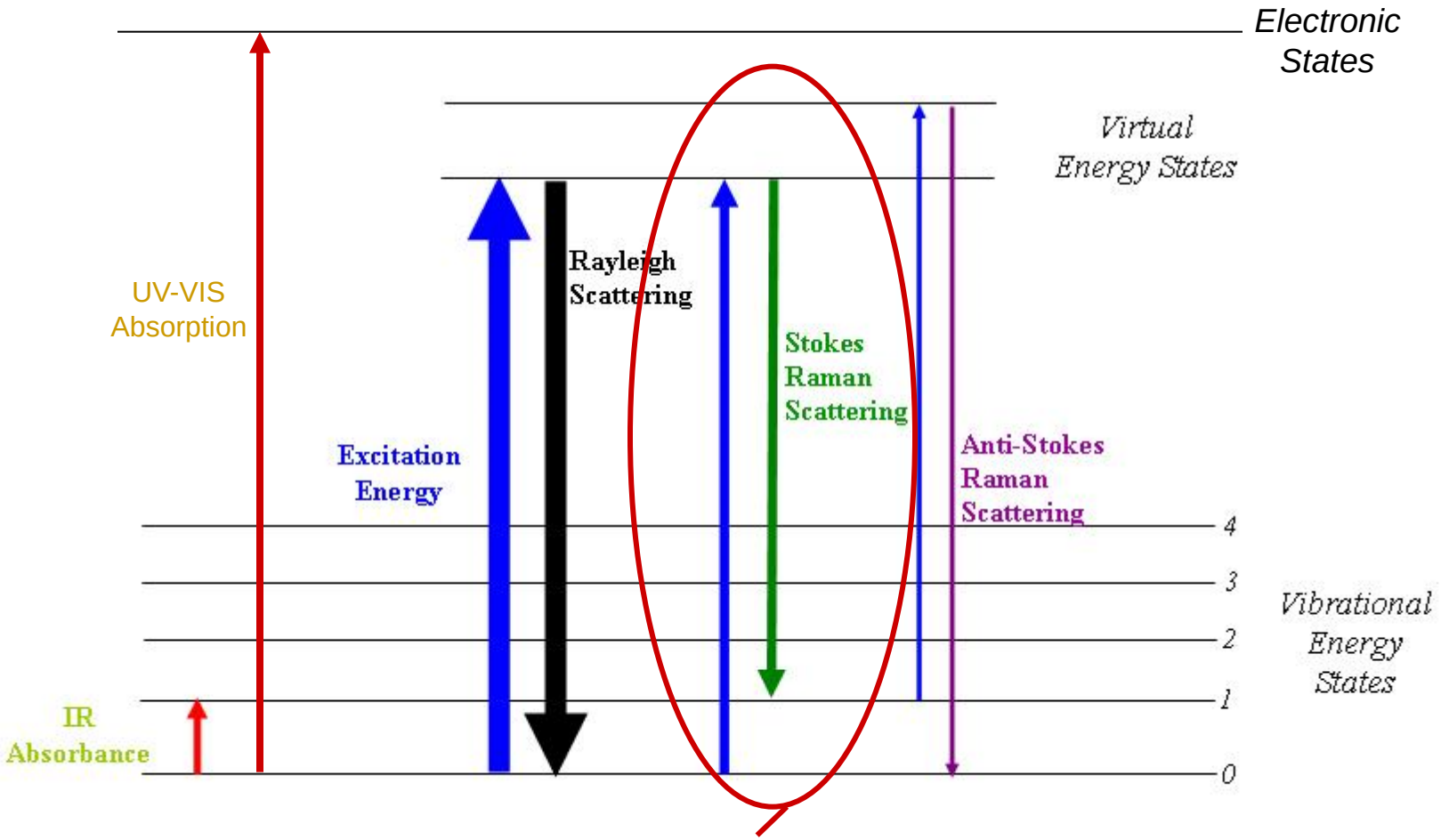
Raman Spectroscopy: Basic Concept



If light is not absorbed… the majority of the photons pass through the sample by means of Rayleigh Scattering

http://en.wikipedia.org/wiki/File:Raman_energy_levels.jpg

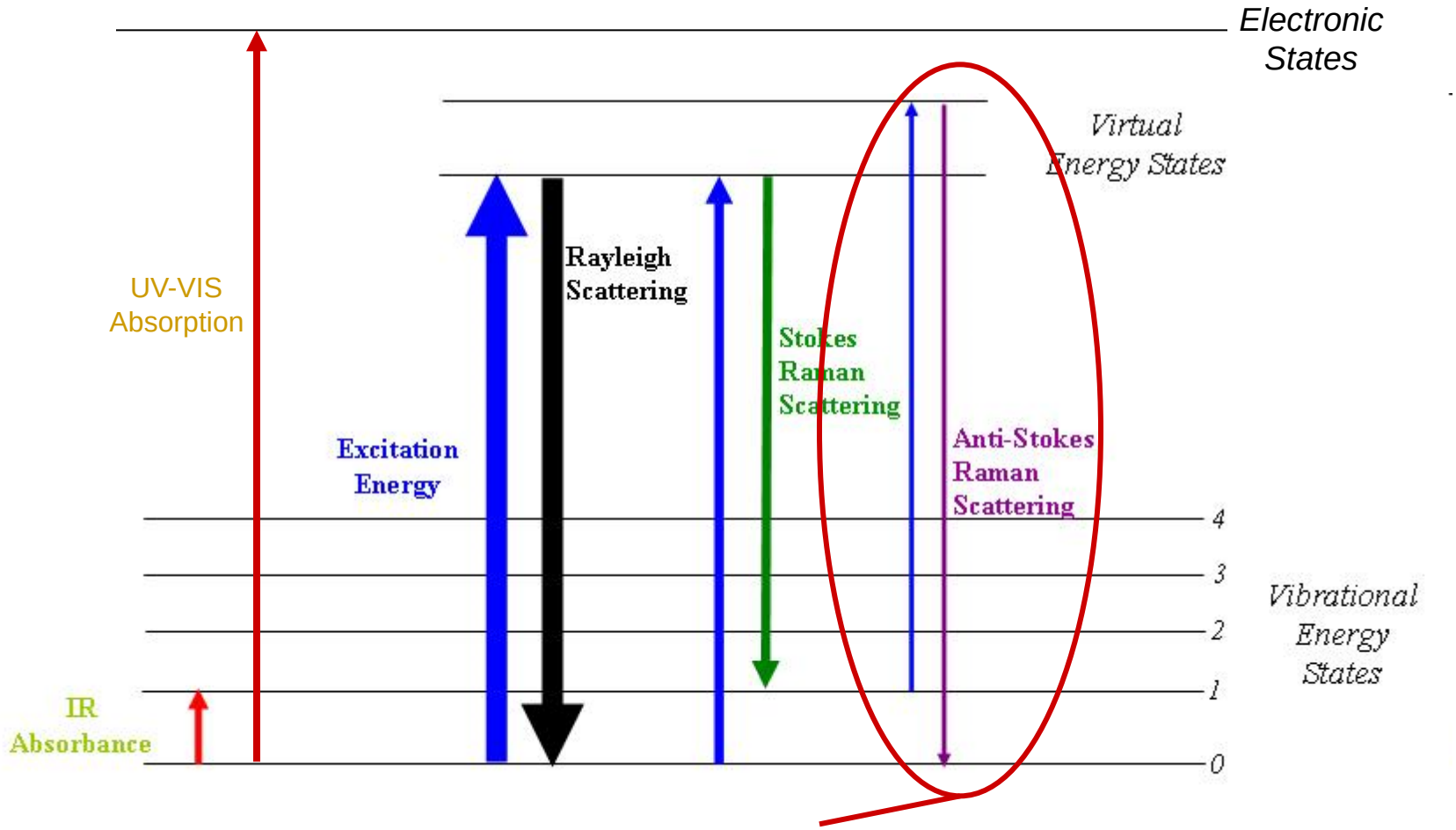
Raman Spectroscopy: Basic Concept



Some of the photons (~1/10,000,000) that are not absorbed will pass through the sample and undergo Stokes Raman Scattering

http://en.wikipedia.org/wiki/File:Raman_energy_levels.jpg

Raman Spectroscopy: Basic Concept



Even fewer photons (temperature dependent) that are not absorbed will pass through the sample and undergo Anti-Stokes Raman Scattering

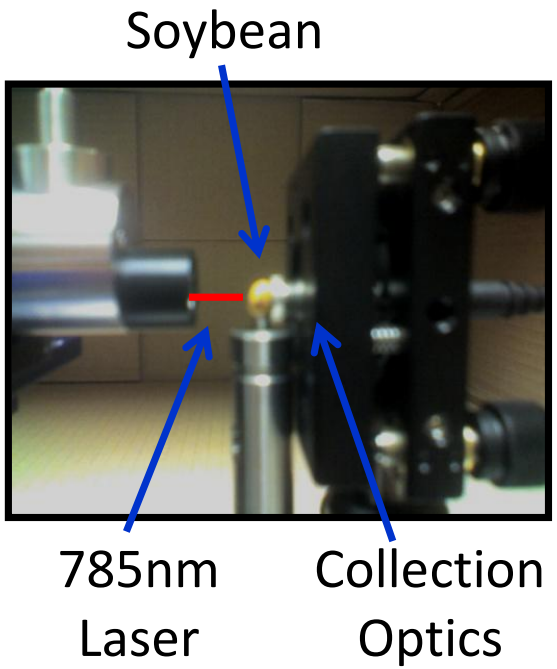
http://en.wikipedia.org/wiki/File:Raman_energy_levels.jpg

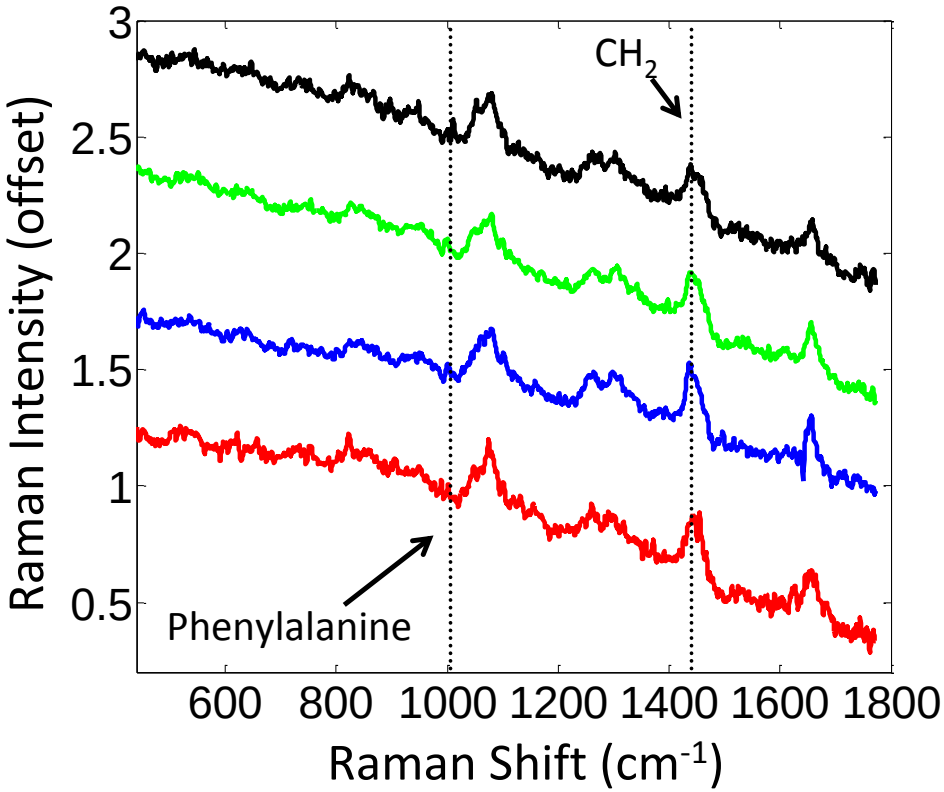
Project Background

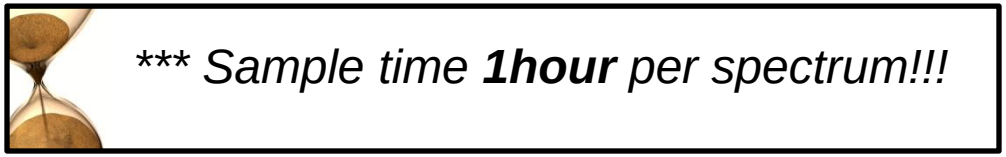
Can Raman Spectroscopy be Utilized for Soybeans?

What Value Can it Measure?

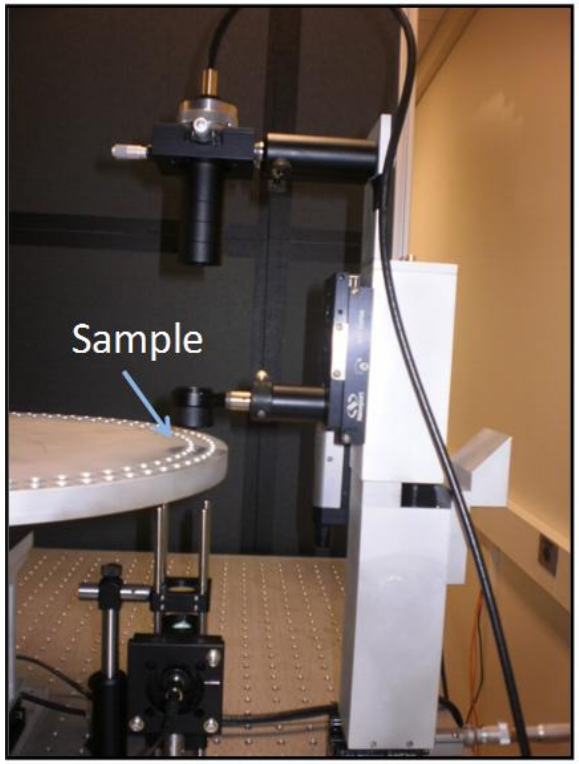
Can we even get a signal?



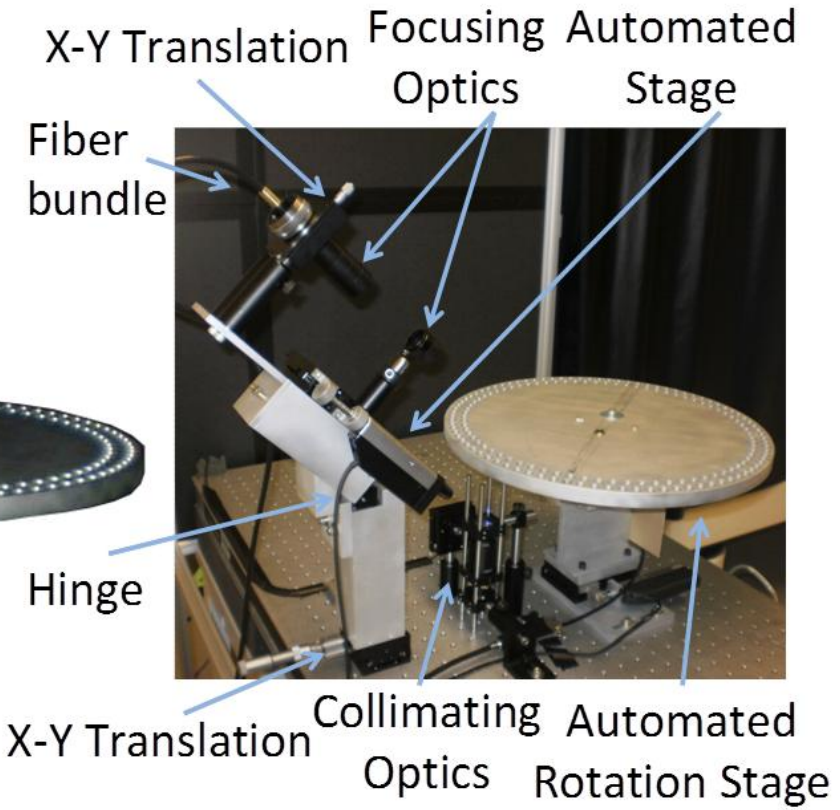




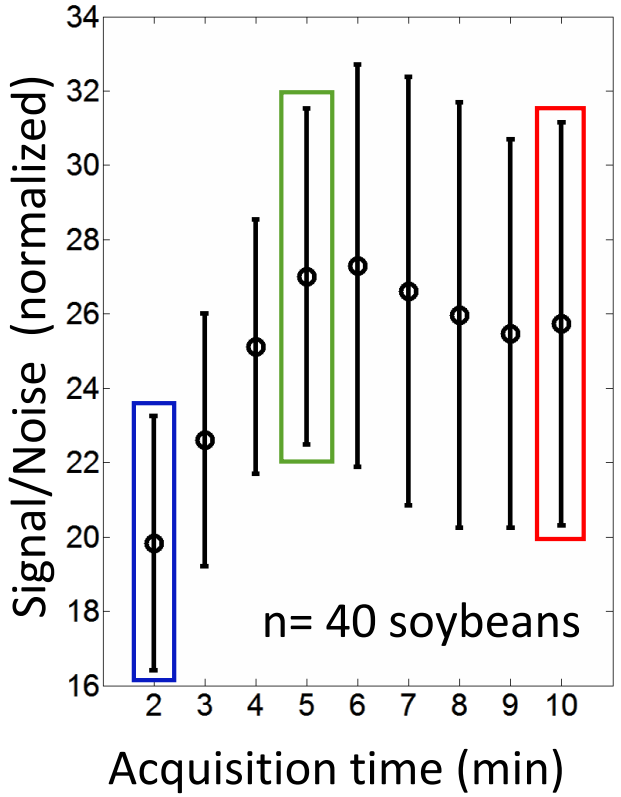
Single Soybean Instrumentation

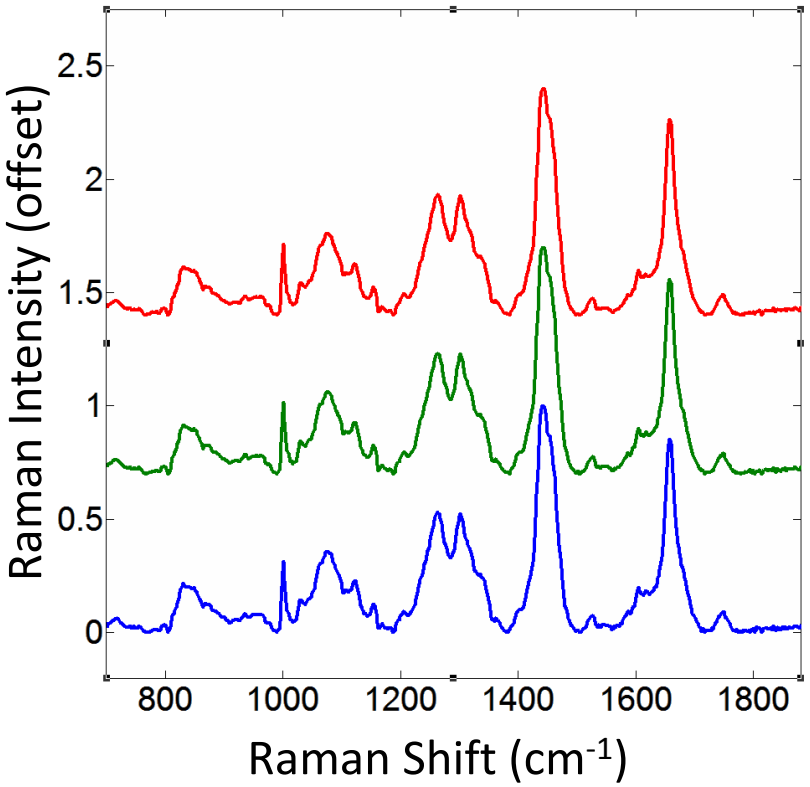






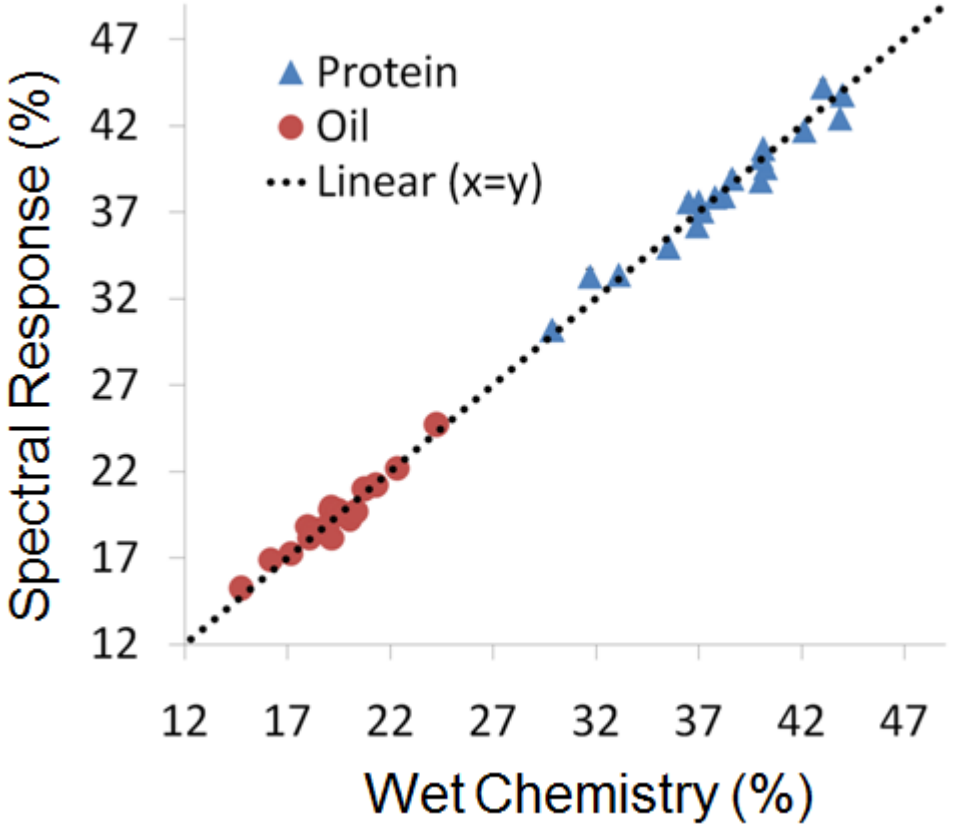
Measurement Times





*** *We could probably get away with a 60sec or even 30sec acquisition*

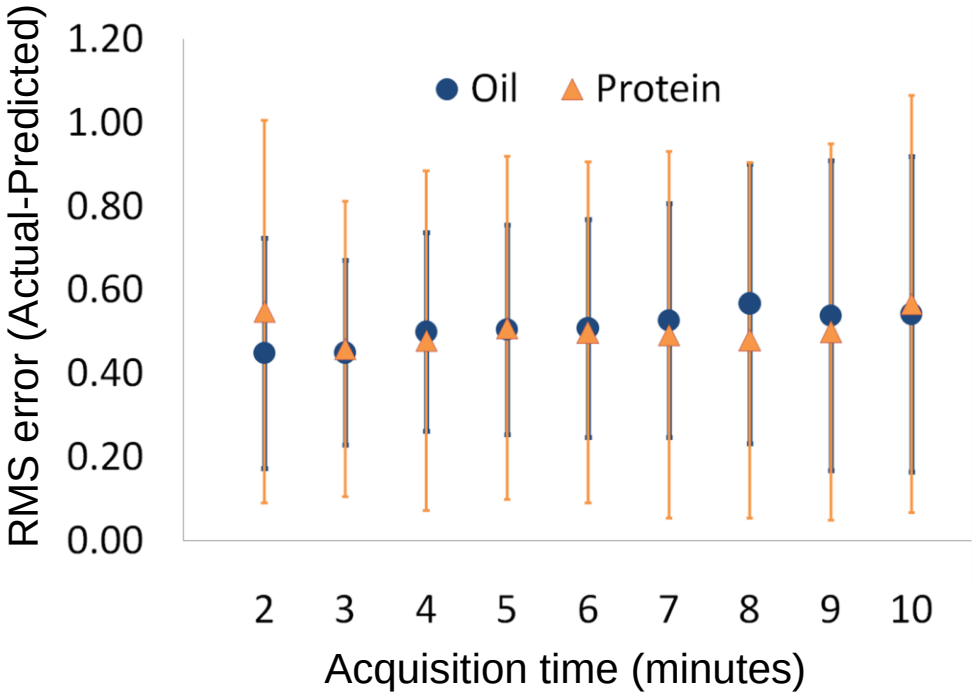
Correlation Plot

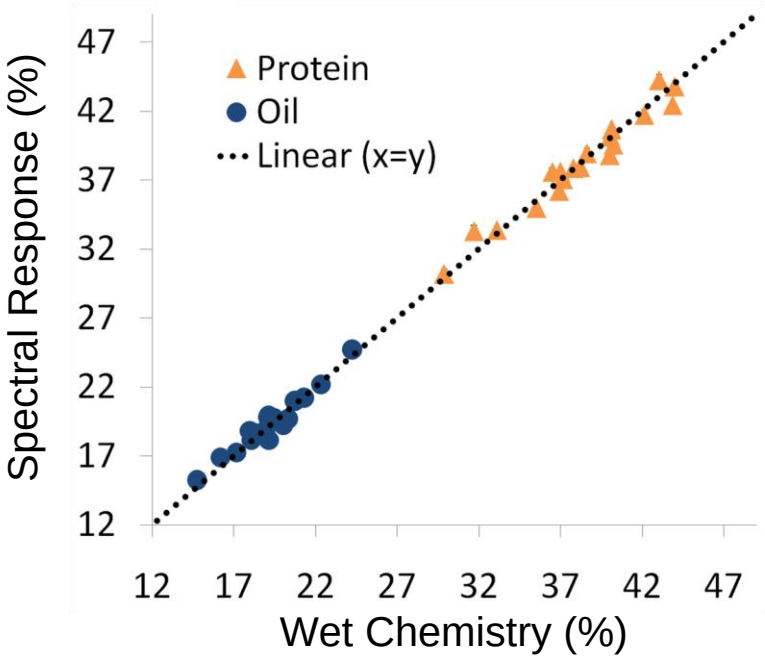




Signal Soybean Measurements

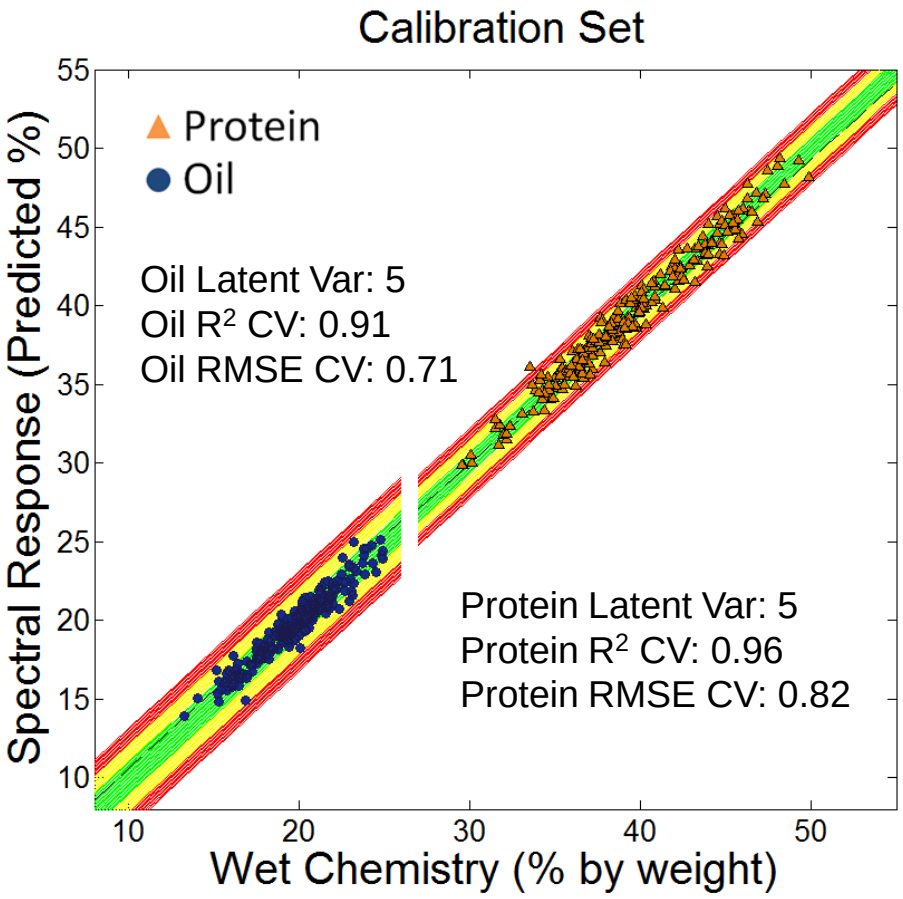
Measurement Times





*** *PLS Leave one out cross-validation 20 protein and 20 oil*

Soybean Measurements

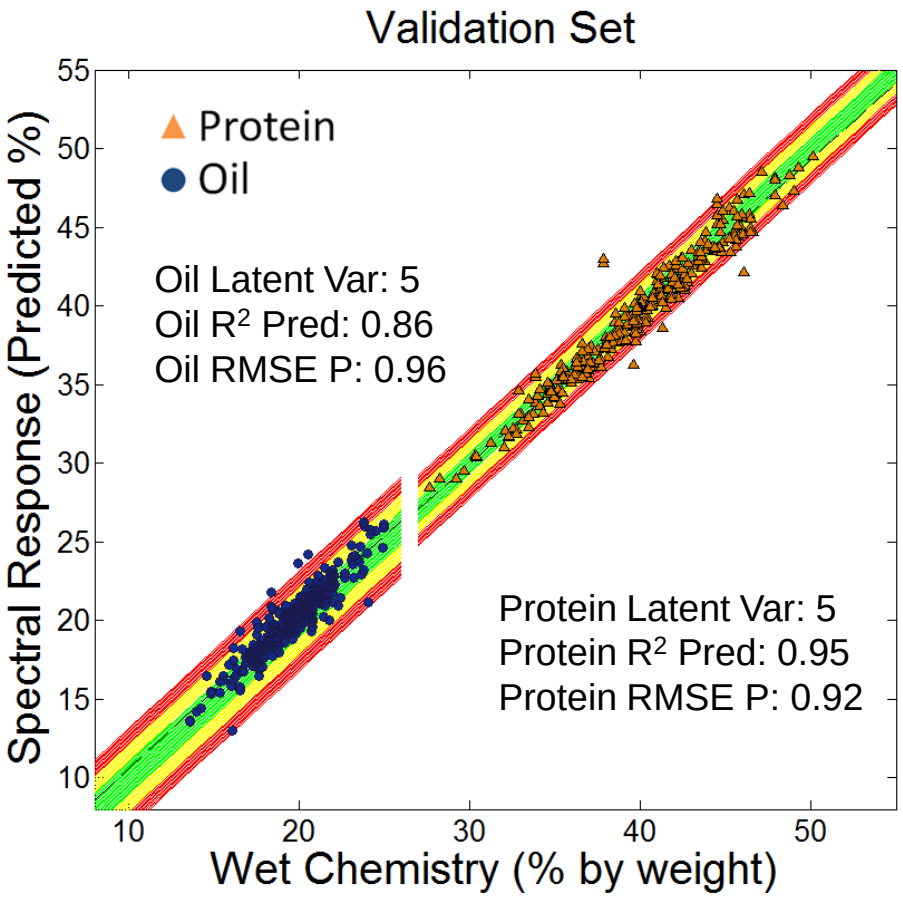


236 soybeans for **oil** calibration
237 soybeans for **protein** calibration



Signal Soybean Measurements

Soybean Measurements

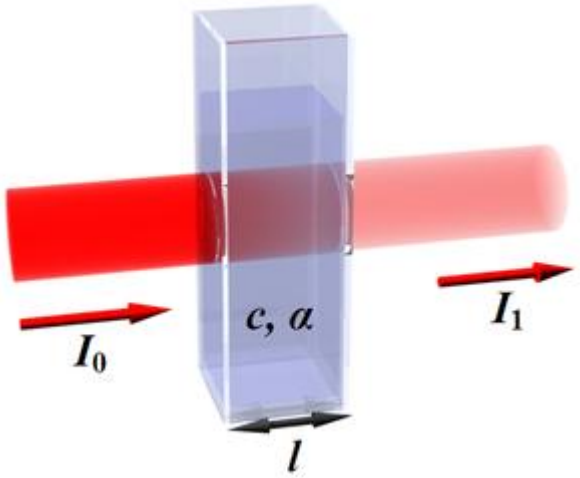


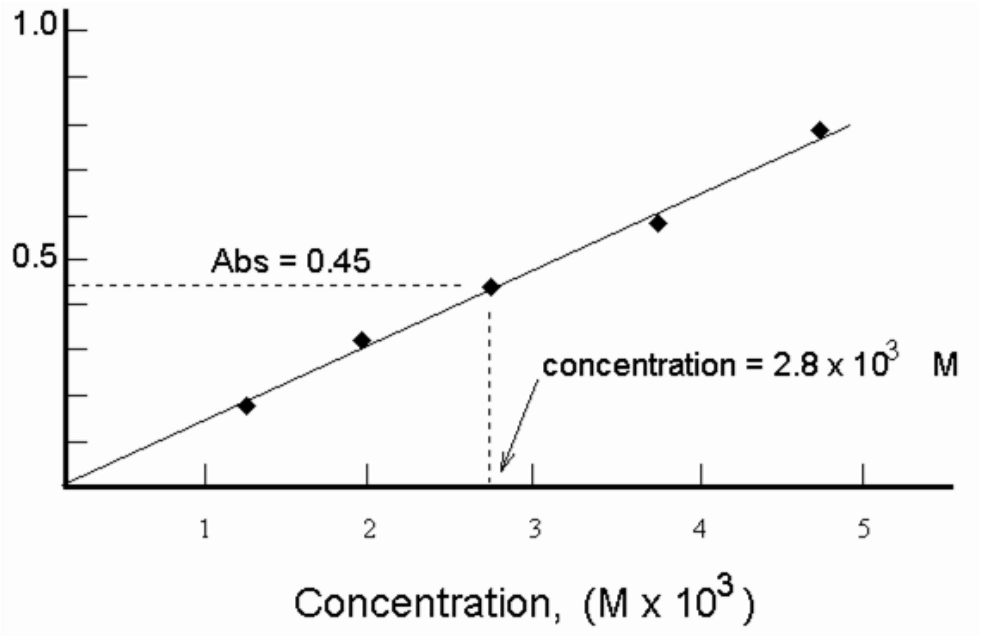
298 soybeans for **oil** validation
299 soybeans for **protein** validation



Signal Soybean Measurements

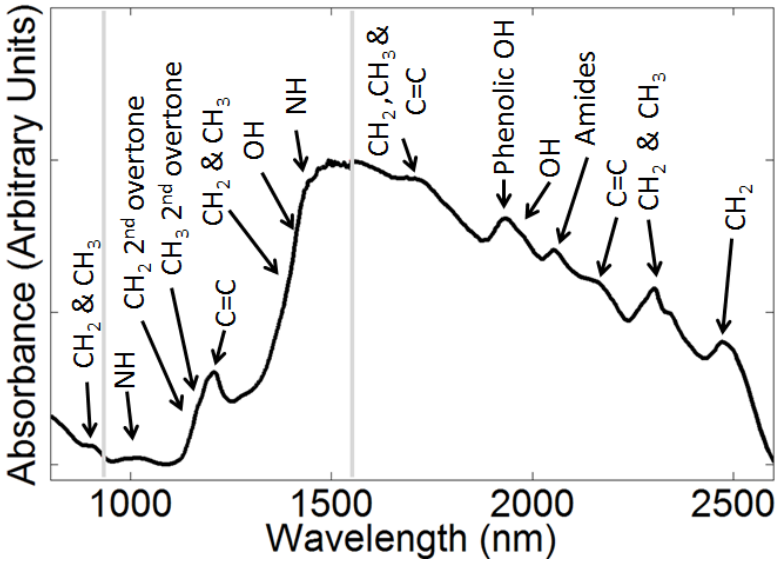
Beer's Law





NIR, mid-IR, and Raman all follow Beer's Law

NIR –vs.- IR –vs.- Raman



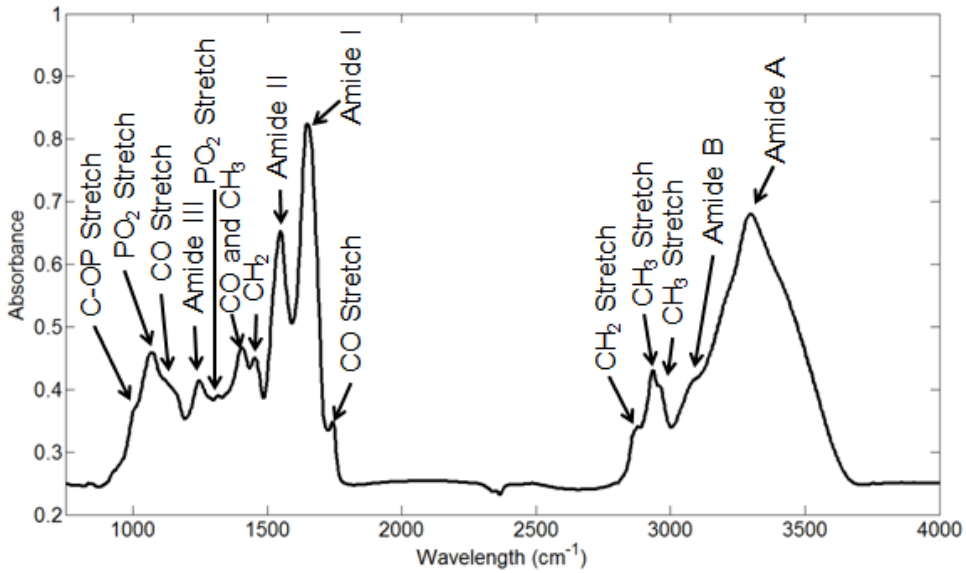
- NIR Light tends to scatter more than it is absorbed so you have greater penetration depth

-The spectral information arises from broad spectral overtones and as a result it is difficult to assign spectral features to specific chemical components

- Light scattering tends to be a problem, corrections are needed



NIR –vs.- IR –vs.- Raman



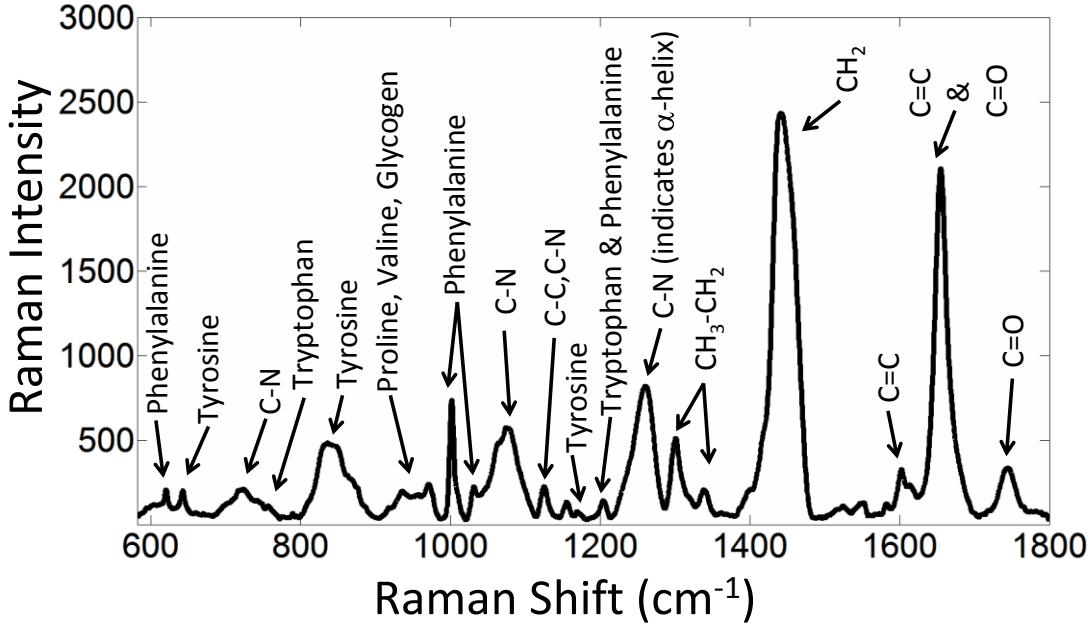
- IR Light tends to absorb more than it scatters so you have very little penetration depth

-The spectral information arises from specific vibration arising from molecular functional groups and as a result you can assign spectral features to specific chemical components

- Sample thickness/preparation tends to be the biggest difficulty with IR spectroscopy



NIR –vs.- IR –vs.- Raman



-Raman can use any single wavelength of light

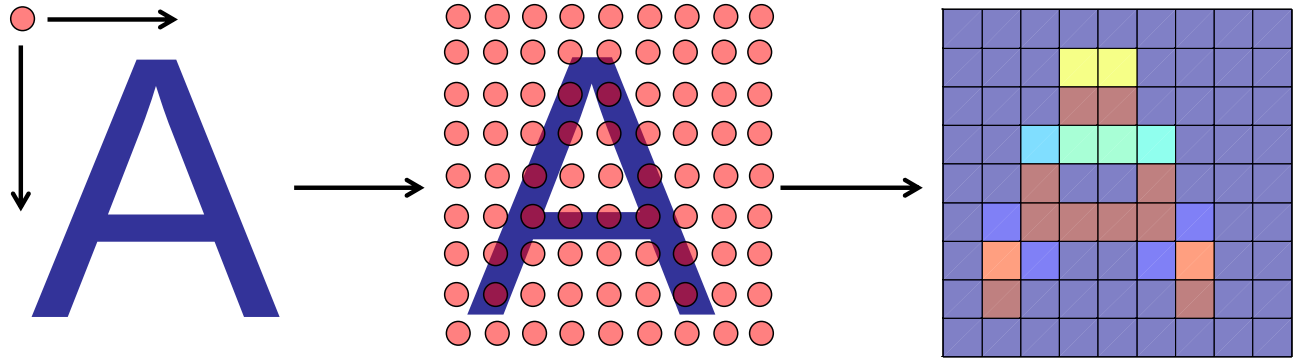
-Raman has narrow band and as a result achieves very high chemical sensitivity (band can be assigned to specific molecular groups)

- Quantum efficiency is low for Raman spectroscopy (longer acquisition times)

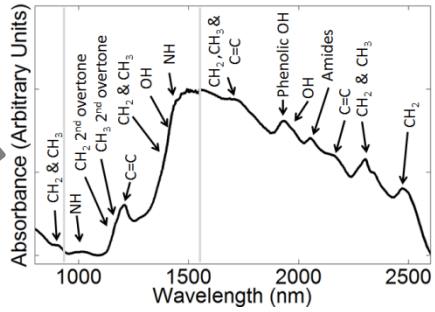


Spectral Imaging

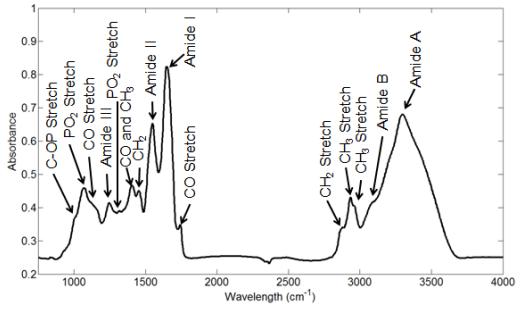
Conventional Mapping: 81 measurements



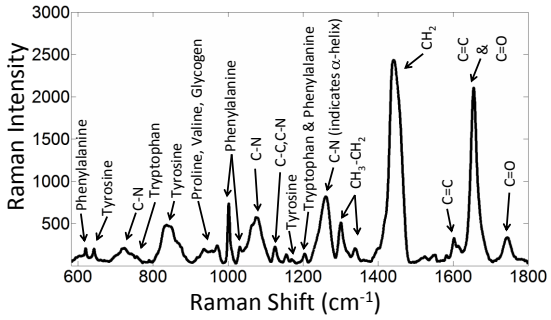
This is a 3 dimensional data set where x and y are spatial dimensions and for the third each pixel has a spectrum that can show sample Chemistry



or



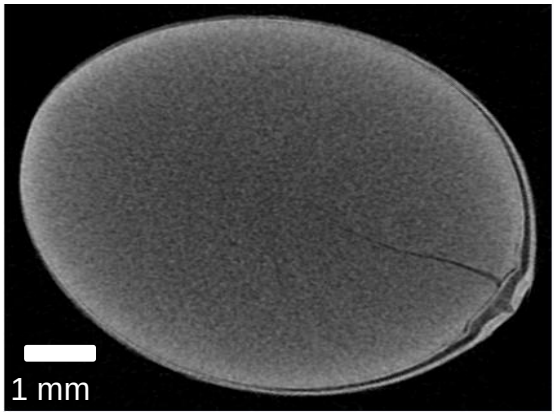
or



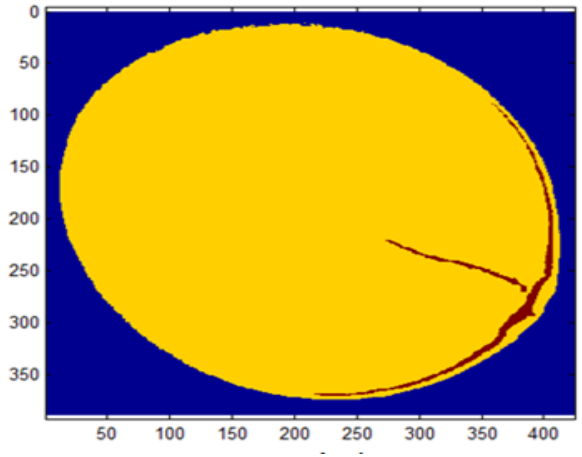
NIR ***Mid-IR*** ***Raman***

Monte Carlo Simulation

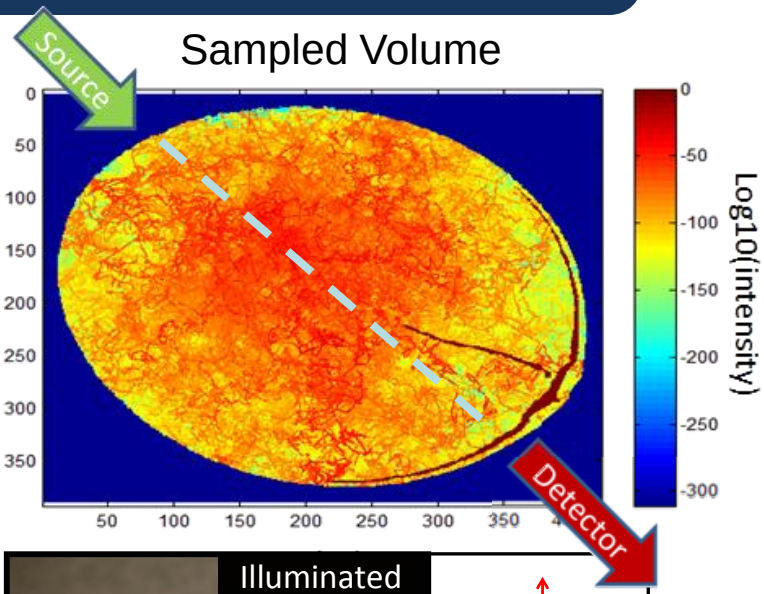
CT Scan of a Soybean

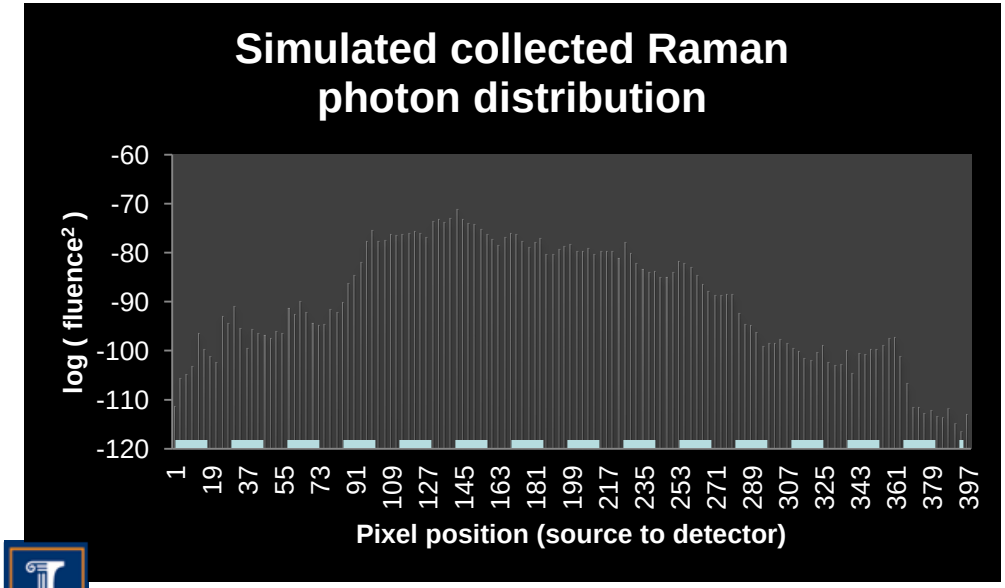


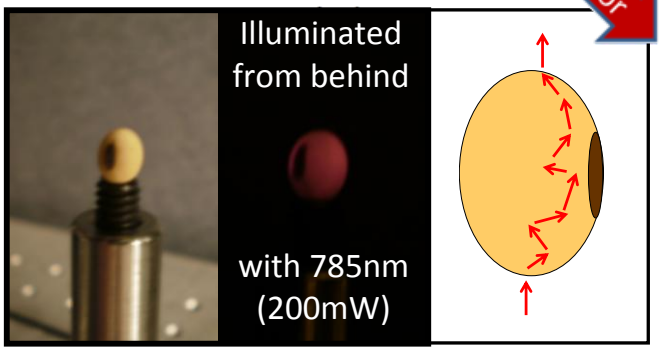
mesh



Sampled Volume

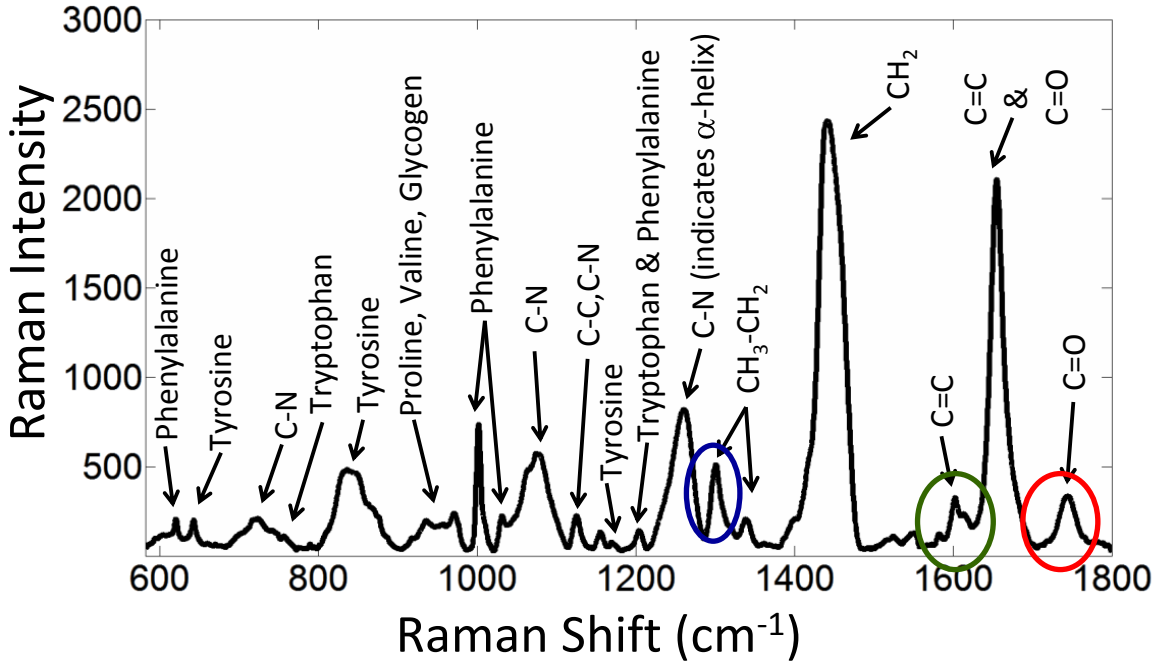


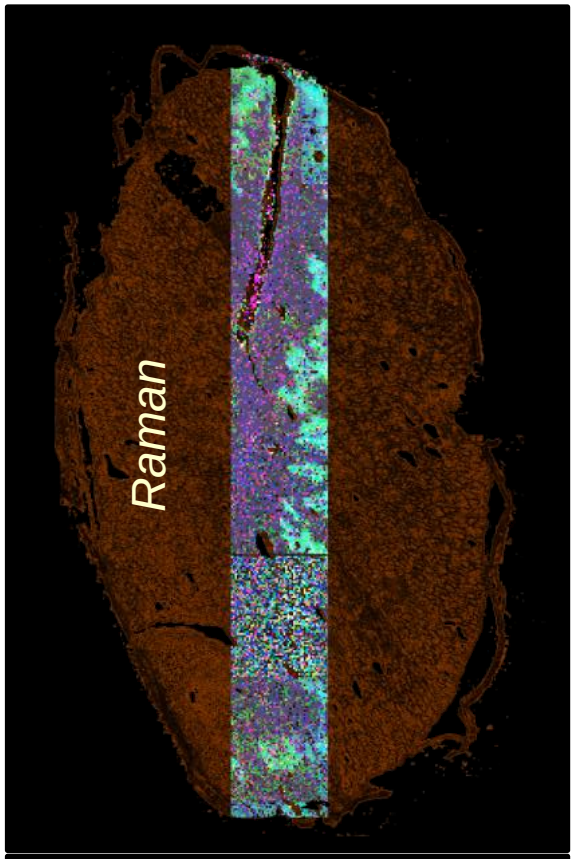




****homogenized by transmission Raman spectroscopy (samples the bulk of the soybean as opposed to the surface)*

Chemical Distribution



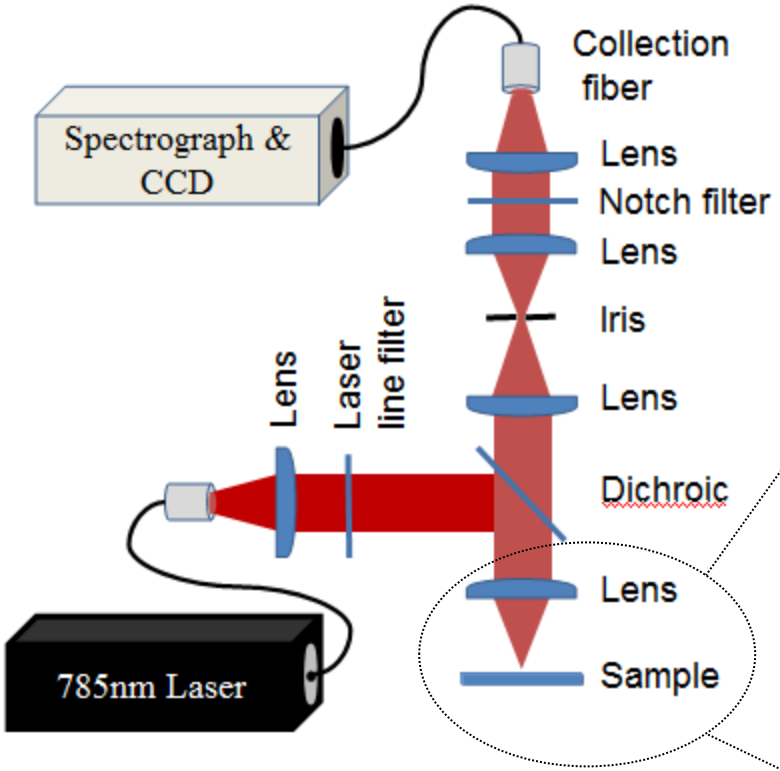


C=O C=C CH_2,CH_3

Phenylalanine

***Serial soybean sections cut by microtome to 5μm thick

Conventional Raman Spectroscopy



Works really well for many, many samples… but not so well for others… depends what you are trying to do:



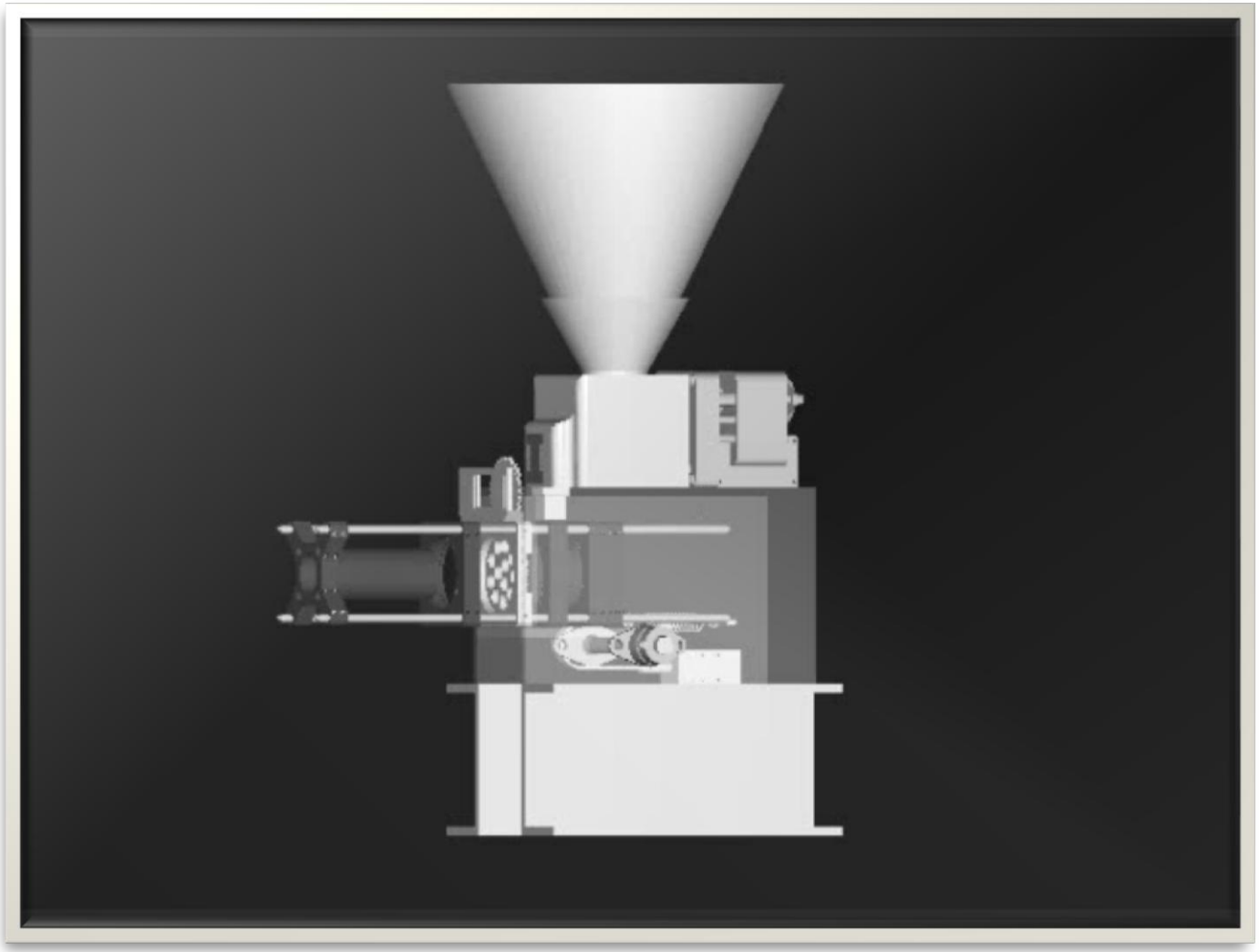


N. Everall, *Appl.Spectrosc.* 63, 245A (2009) N. Everall, Appl. Spectrosc. 62, 591 (2008). M. J. Pelletier, Appl. Spectrosc. 63, 591 (2009)

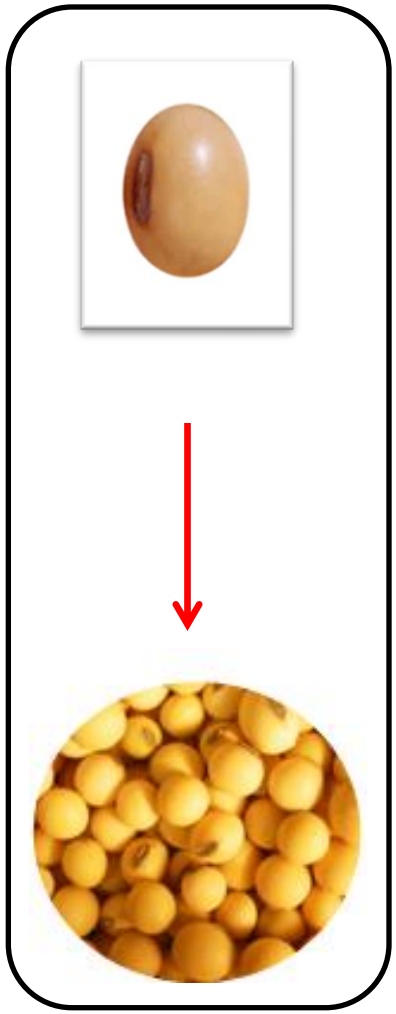
Single Soybean to Bulk







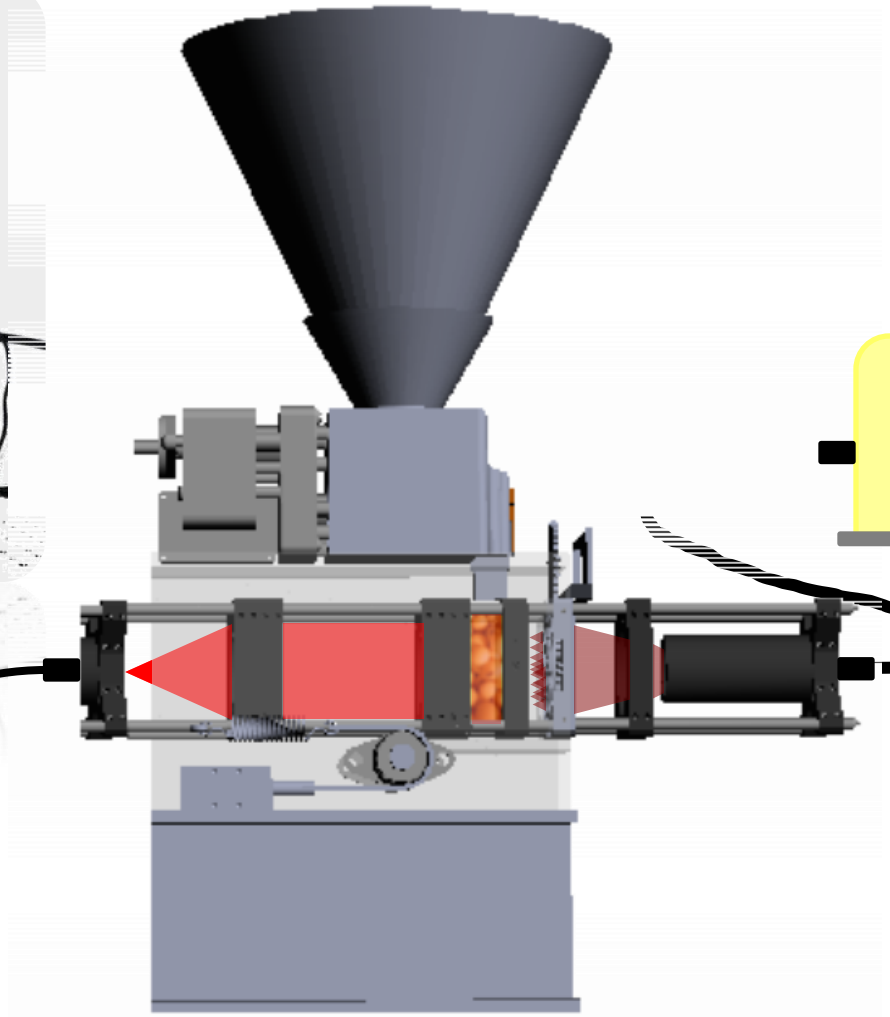
Single Soybean to Bulk





Single Soybean to Bulk





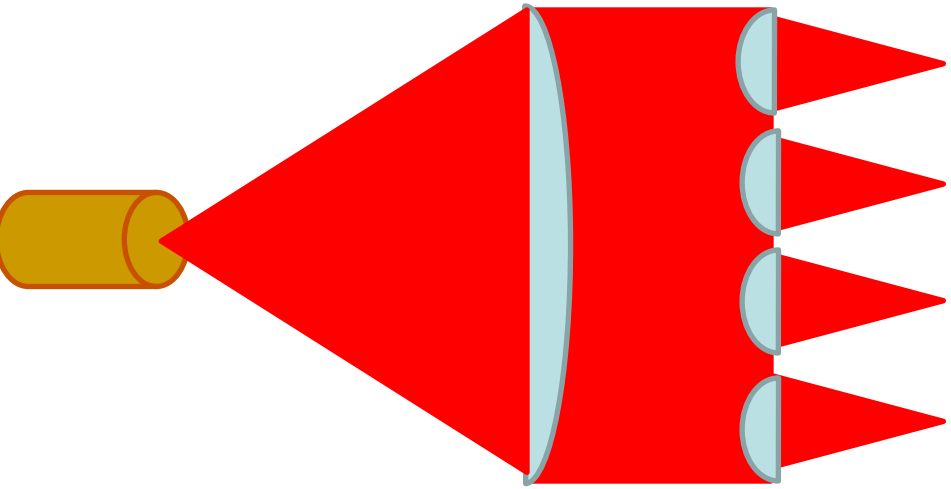
Spectrograph & CCD

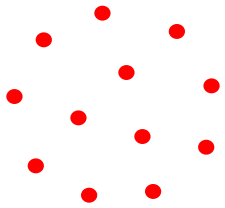
Optics

We need to collect diffuse light over a large area with high efficiency… *Lens-let Array!!!*



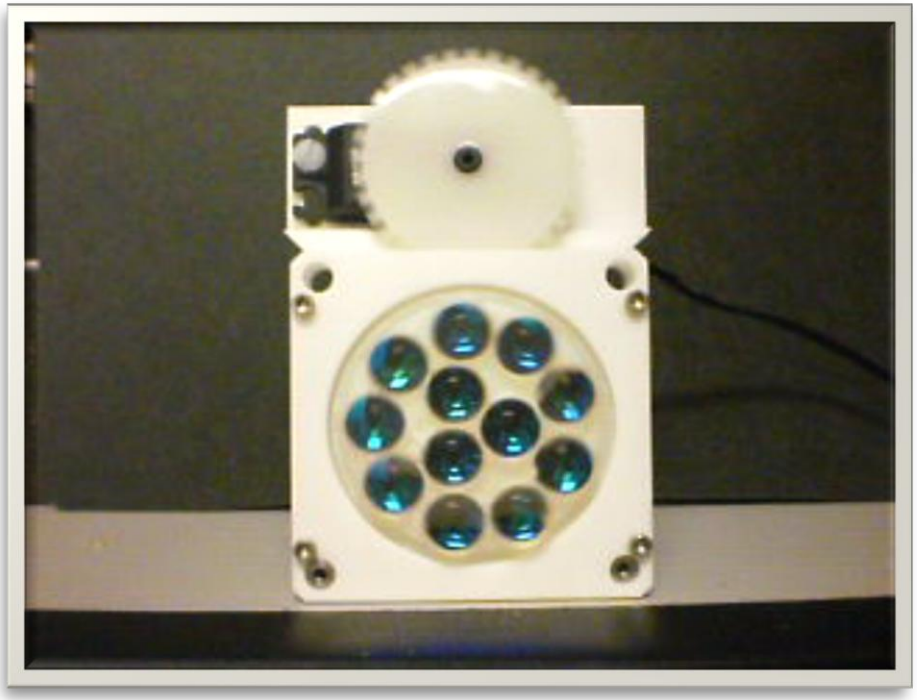
12 Lenses with f/# of 2

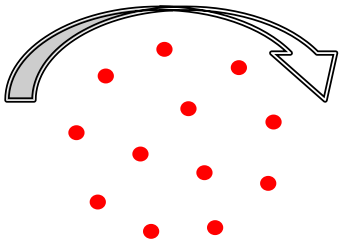




So we are collecting from 12 locations on the sample

Optics

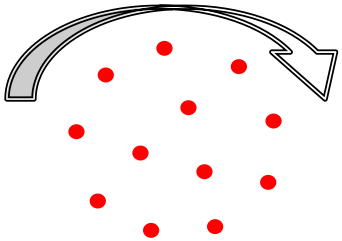




So we are collecting from 12 locations on the sample

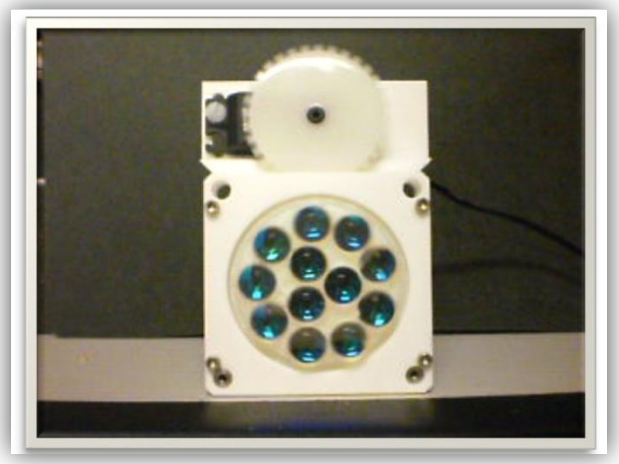
Optics

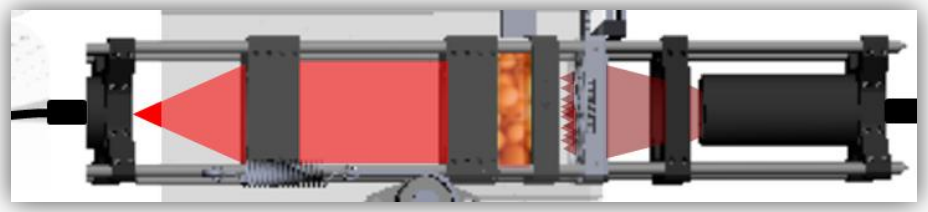




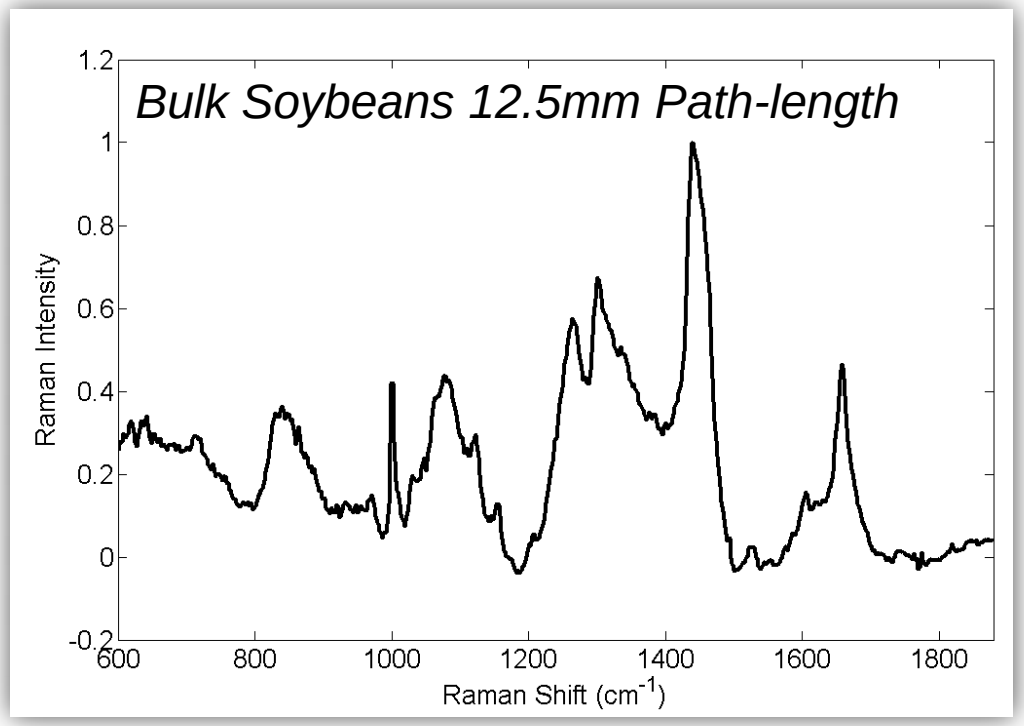
So we are collecting from 12 locations on the sample

Transmission Raman Signal





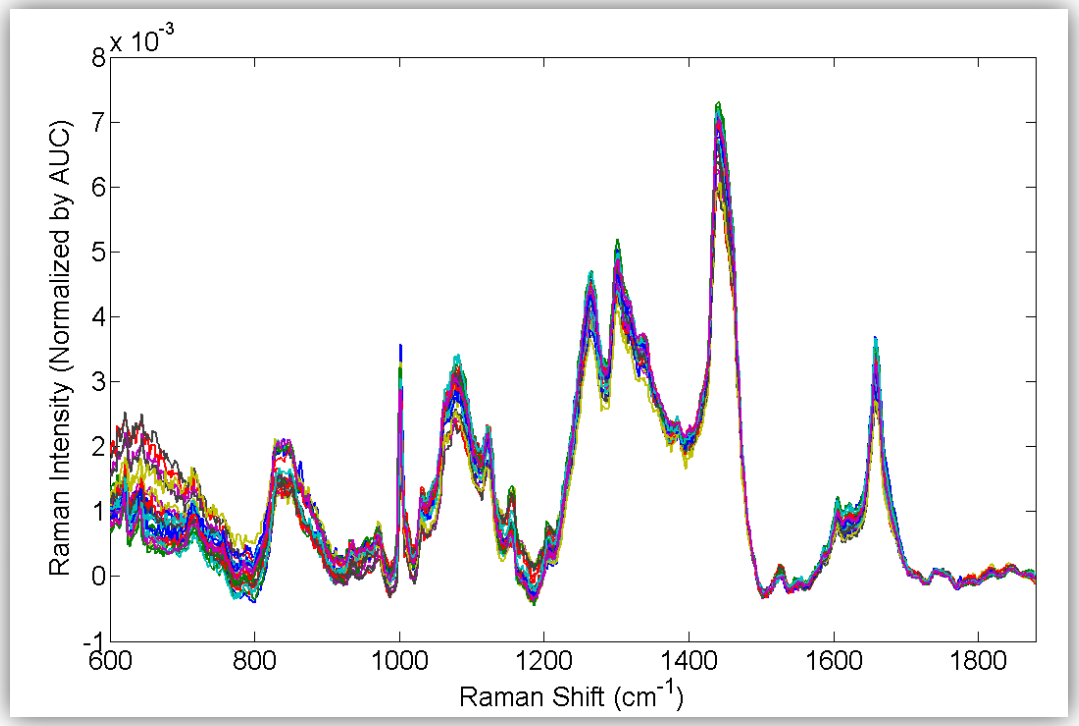




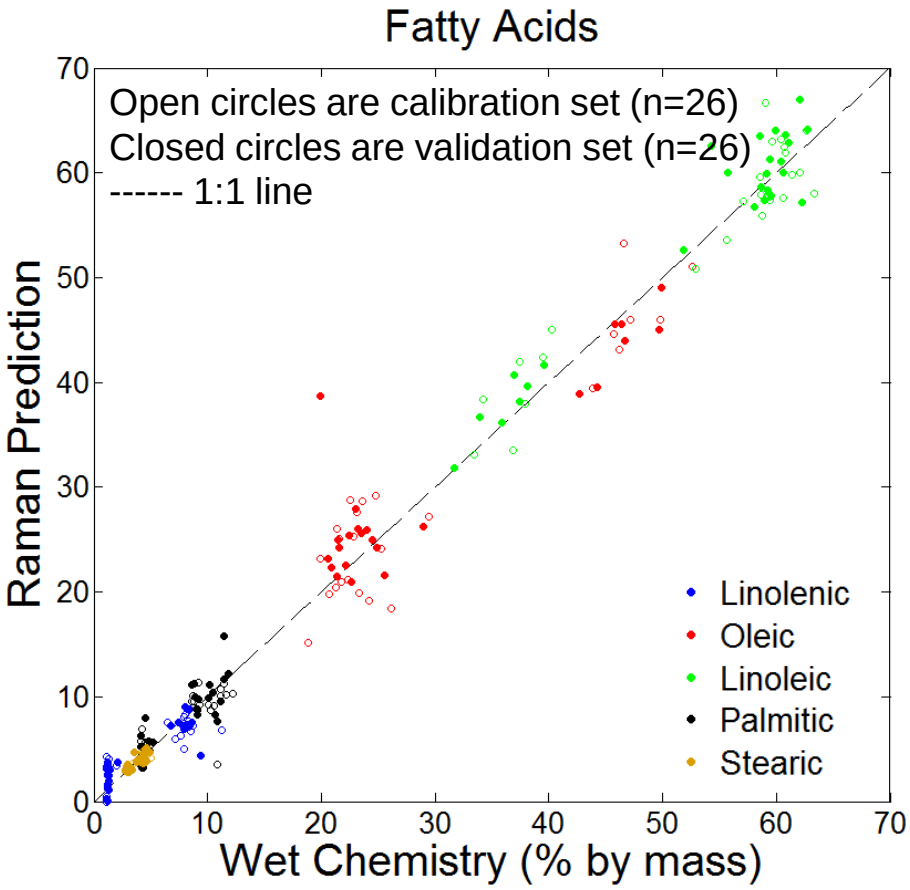
The Experiment

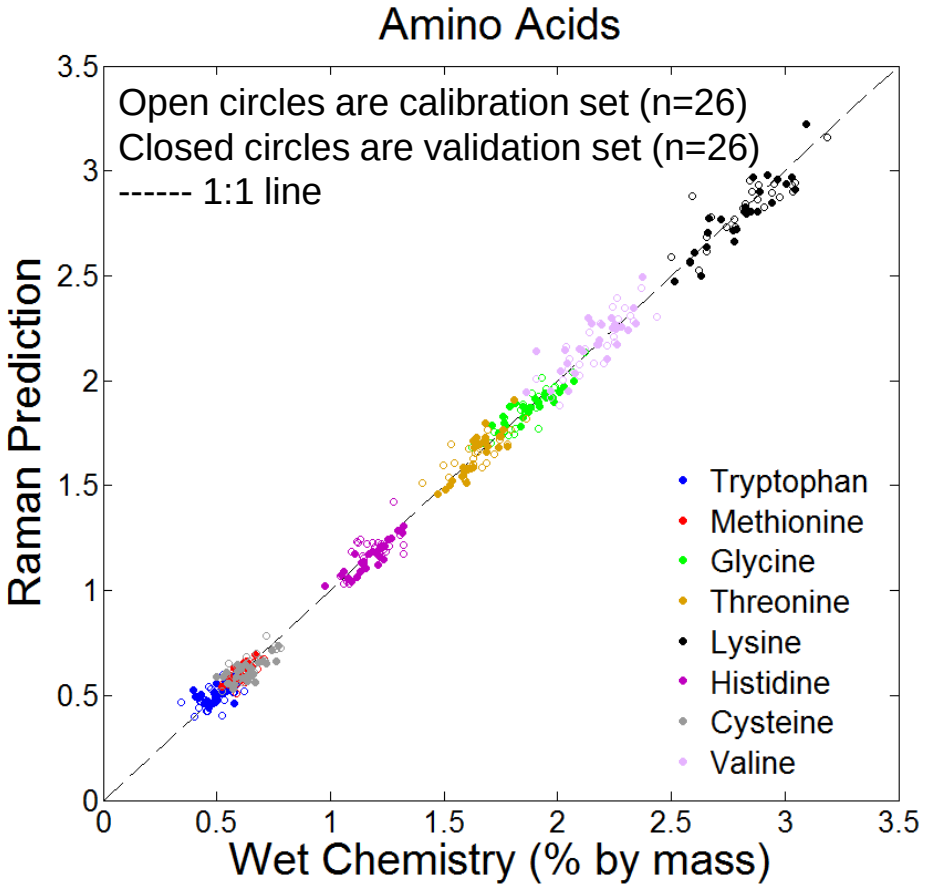


-52 soybean varieties
-300mW of laser power
-5 runs at 10min/run per variety
-Wet chemistry for Amino Acids and Fatty Acids



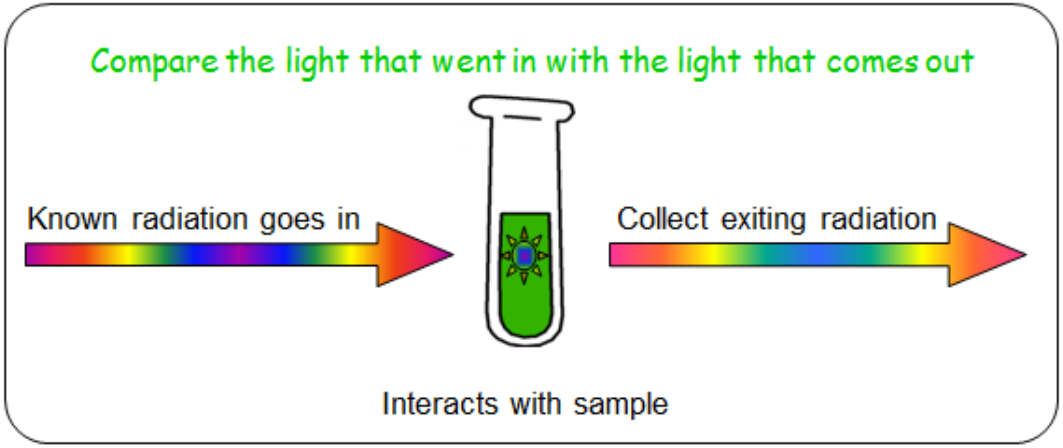
Results

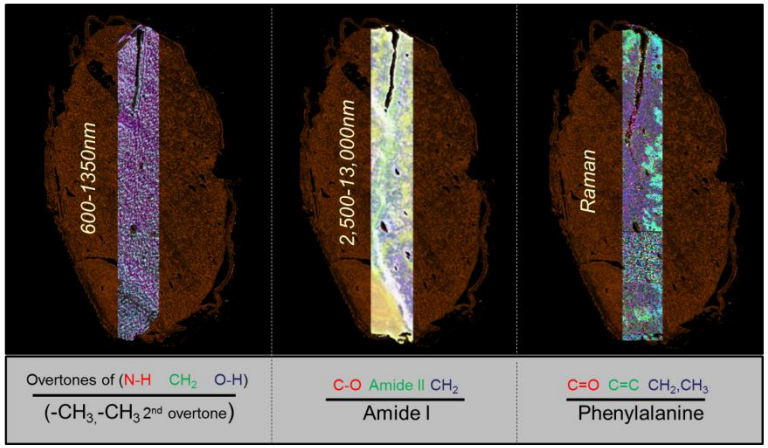




** Calibration uses a PLS model *** Still working on Sugars

Summary


Compare the light that went in with the light that comes out
Known radiation goes in
Collect exiting radiation
Interacts with sample


600-1350nm
2,500-13,000nm
Raman
Overtones of (N-H CH2 O-H)
(-CH3,-CH3 2nd overtone)
C-O Amide II CH2
Amide I
C=O C=C CH2,CH3
Phenylalanine


ILLINOIS



Thank you

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