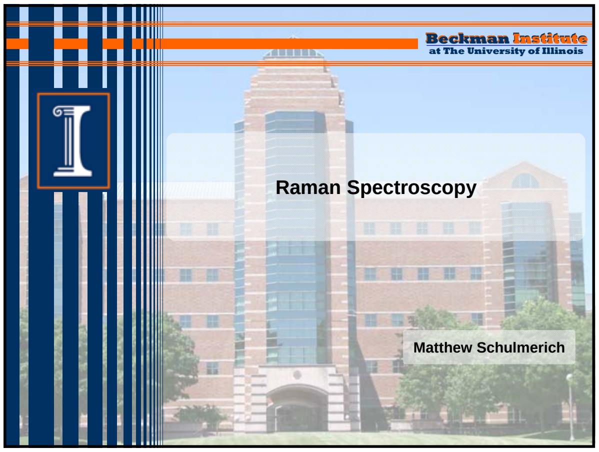
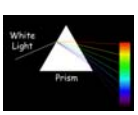


Overview

1) What is spectroscopy?
2) What is Raman Spectroscopy?
3) What are the basics of Raman Instrumentation?
5) What are the advantages and Disadvantages of Raman Spectroscopy?
6) Experimental Approaches
   > Coupling Raman to a Microscope
   > Raman mapping/imaging
   > Less traditional Raman measurements

What is Spectroscopy?

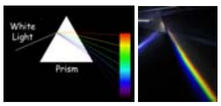
A Rainbow is a visible spectrum



Spectroscopy is converting light into information:

What is Spectroscopy?

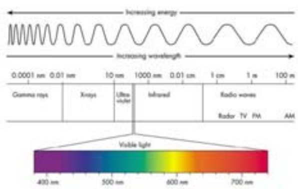
A Rainbow is a visible spectrum



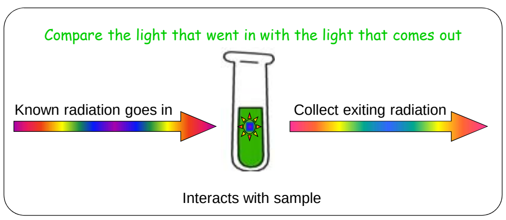
Spectroscopy is converting light into information:

-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.

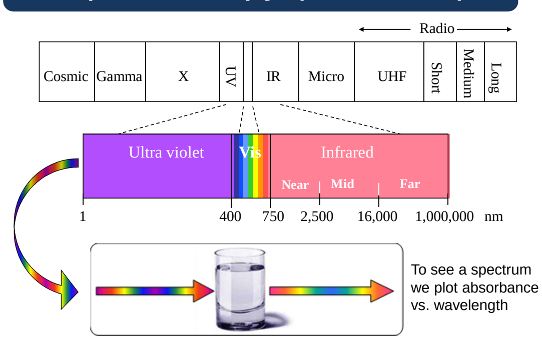


Spectroscopy generalization



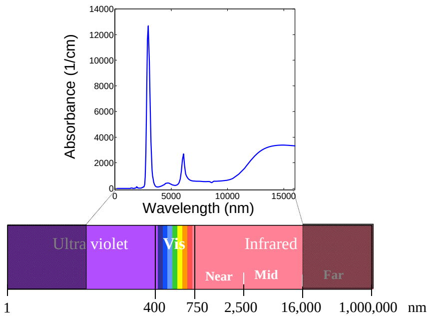
A general definition of spectroscopy is the study of the interaction between radiation and an analyte as a function of wavelength.

Spectroscopy (Radiation)



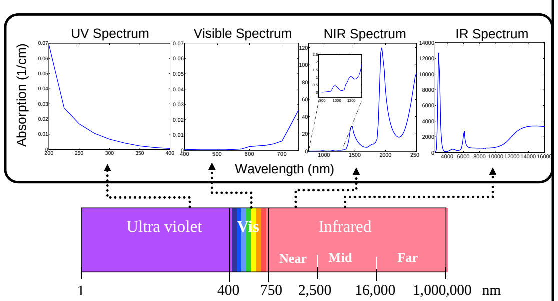
To see a spectrum we plot absorbance vs. wavelength

Spectroscopy of liquid water



Appl. Opt., 12, 555--563, (1973)

Spectroscopy of liquid water

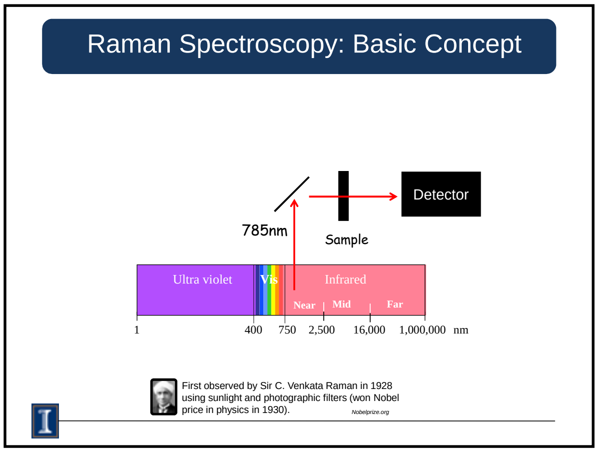


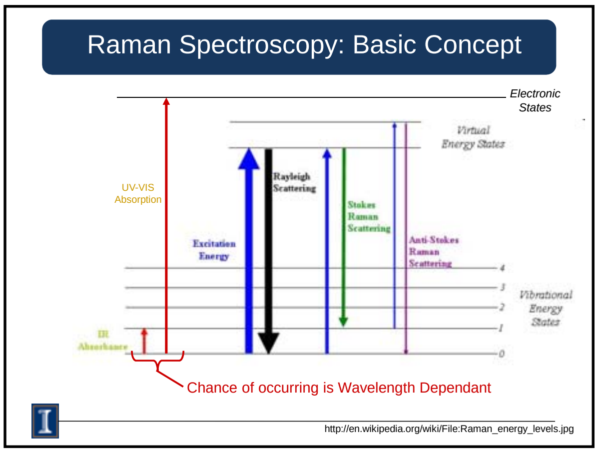
Spectroscopy Generalization

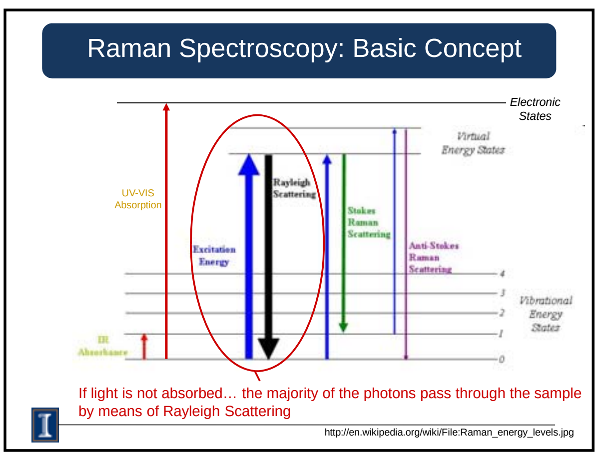
Reasons for Light absorption:

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

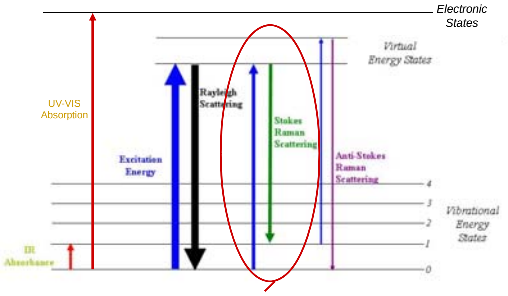
Infrared and Raman Spectroscopy: 'Vibrational spectroscopy', spectral bands occur as a result of molecular vibrations


Raman Spectroscopy: Basic Concept
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
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
Electronic States
Virtual Energy States
UV-VIS Absorption
Rayleigh Scattering
Stokes Raman Scattering
Excitation Energy
Anti-Stokes Raman Scattering
Vibrational Energy States
IR Absorbance
Chance of occurring is Wavelength Dependant
http://en.wikipedia.org/wiki/File:Raman_energy_levels.jpg


Raman Spectroscopy: Basic Concept
Electronic States
Virtual Energy States
UV-VIS Absorption
Rayleigh Scattering
Stokes Raman Scattering
Excitation Energy
Anti-Stokes Raman Scattering
Vibrational Energy States
IR Absorbance
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

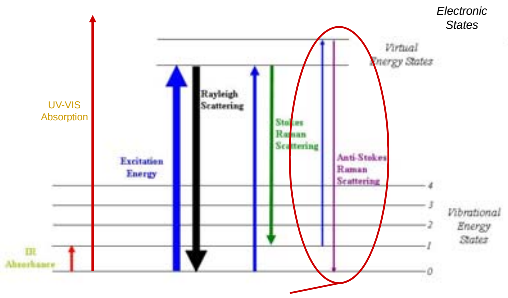
Raman Spectroscopy: Basic Concept



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

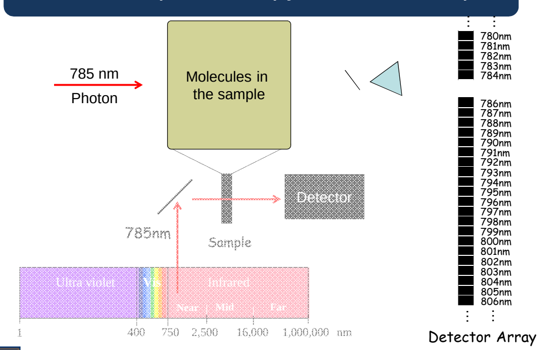
Raman Spectroscopy: Basic Concept

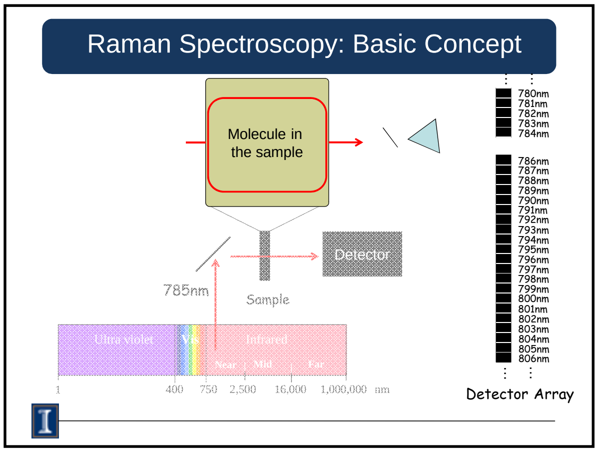


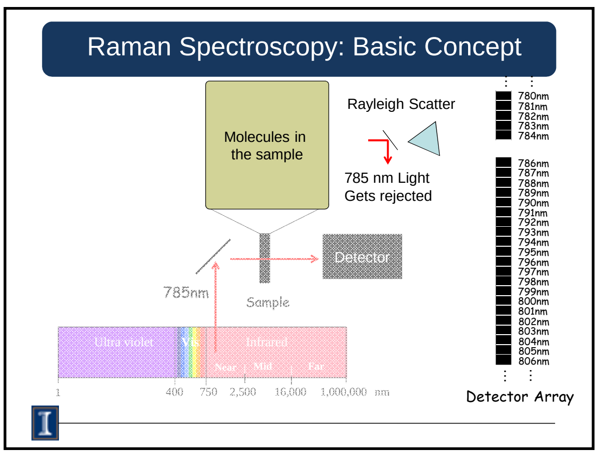
Even fewer photons (temperature dependant) that are not absorbed will pass through the sample and undergo Anti-Stokes Raman Scattering

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

Raman Spectroscopy: Basic Concept




Raman Spectroscopy: Basic Concept
Molecule in the sample
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000 nm
780nm
781nm
782nm
783nm
784nm
786nm
787nm
788nm
789nm
790nm
791nm
792nm
793nm
794nm
795nm
796nm
797nm
798nm
799nm
800nm
801nm
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
Molecules in the sample
Rayleigh Scatter
785 nm Light Gets rejected
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000 nm
780nm
781nm
782nm
783nm
784nm
786nm
787nm
788nm
789nm
790nm
791nm
792nm
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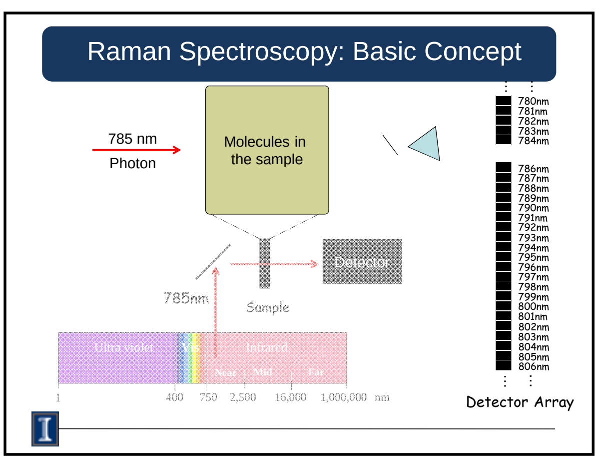
797nm
798nm
799nm
800nm
801nm
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
785 nm Photon
Molecules in the sample
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000 nm
780nm
781nm
782nm
783nm
784nm
786nm
787nm
788nm
789nm
790nm
791nm
792nm
793nm
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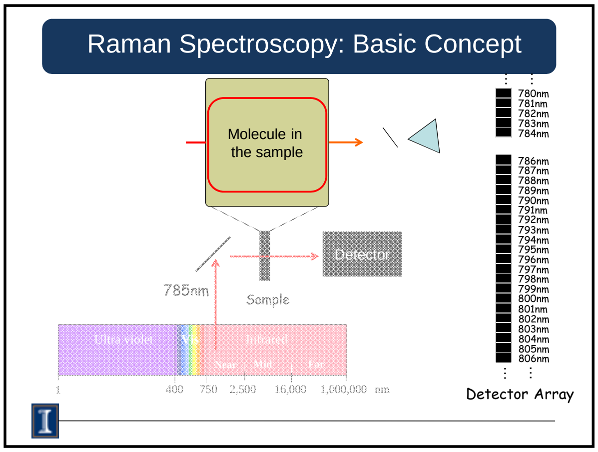
795nm
796nm
797nm
798nm
799nm
800nm
801nm
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
Molecule in the sample
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
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2,500
16,000
1,000,000
nm
780nm
781nm
782nm
783nm
784nm
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800nm
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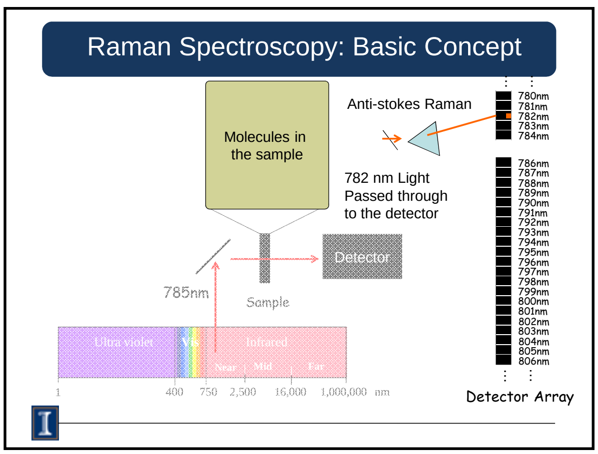
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
Molecules in the sample
Anti-stokes Raman
782 nm Light Passed through to the detector
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
780nm
781nm
782nm
783nm
784nm
786nm
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795nm
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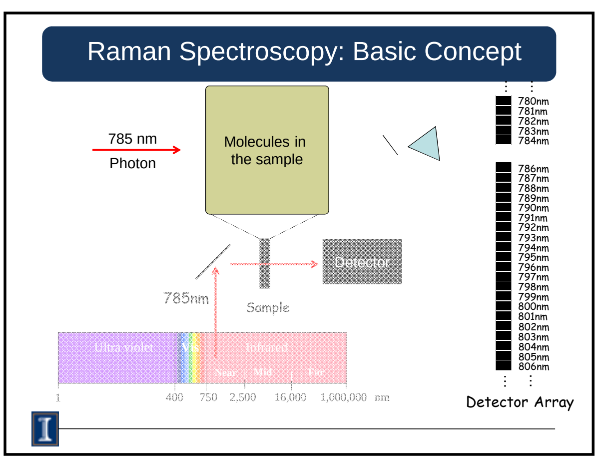
798nm
799nm
800nm
801nm
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
785 nm Photon
Molecules in the sample
Detector
785nm
Sample
Ultra violet
Vis
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
780nm
781nm
782nm
783nm
784nm
786nm
787nm
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790nm
791nm
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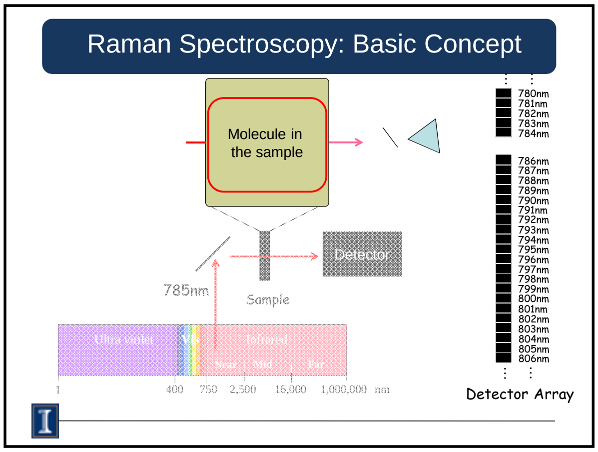
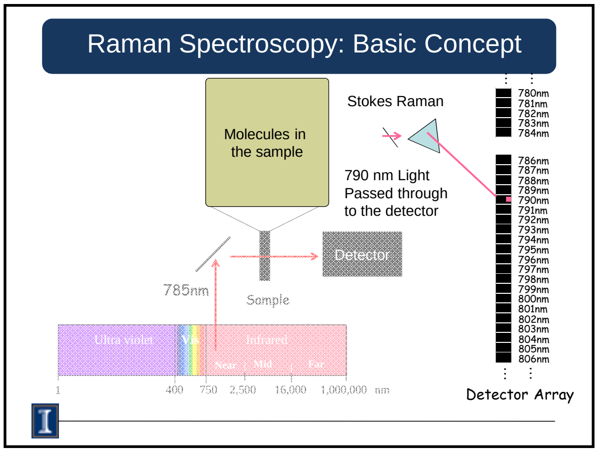
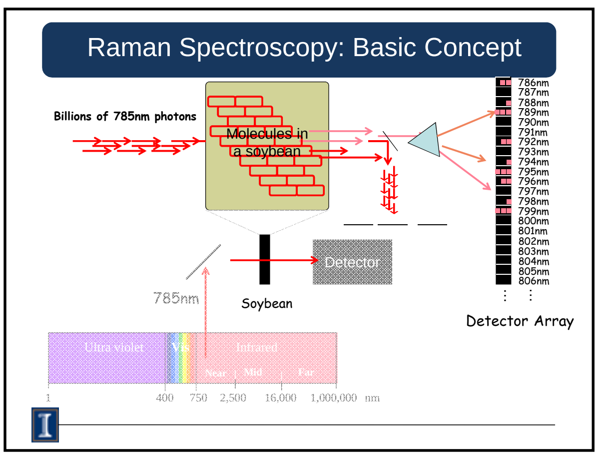
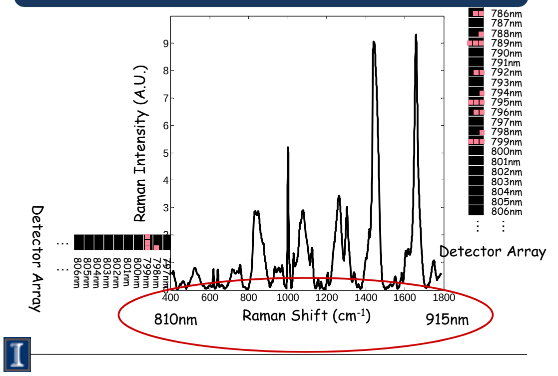
795nm
796nm
797nm
798nm
799nm
800nm
801nm
802nm
803nm
804nm
805nm
806nm
Detector Array


Raman Spectroscopy: Basic Concept
Molecule in the sample
Detector
785nm
Sample
Ultra violet
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
780nm 781nm 782nm 783nm 784nm
786nm 787nm 788nm 789nm 790nm 791nm 792nm 793nm 794nm 795nm 796nm 797nm 798nm 799nm 800nm 801nm 802nm 803nm 804nm 805nm 806nm
Detector Array

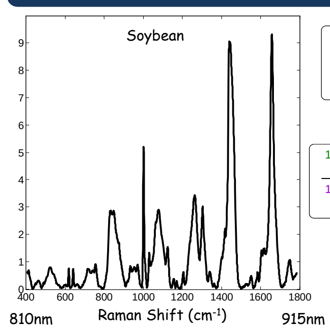

Raman Spectroscopy: Basic Concept
Molecules in the sample
Stokes Raman
790 nm Light Passed through to the detector
Detector
785nm
Sample
Ultra violet
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
780nm 781nm 782nm 783nm 784nm
786nm 787nm 788nm 789nm 790nm 791nm 792nm 793nm 794nm 795nm 796nm 797nm 798nm 799nm 800nm 801nm 802nm 803nm 804nm 805nm 806nm
Detector Array


Raman Spectroscopy: Basic Concept
Billions of 785nm photons
Molecules in a soybean
Detector
785nm
Soybean
Ultra violet
Infrared
Near
Mid
Far
1
400
750
2,500
16,000
1,000,000
nm
786nm 787nm 788nm 789nm 790nm 791nm 792nm 793nm 794nm 795nm 796nm 797nm 798nm 799nm 800nm 801nm 802nm 803nm 804nm 805nm 806nm
Detector Array

Raman Spectroscopy: Basic Concept



Raman Spectroscopy: Wavelength Axis



Excitation Frequency

785nm = 0.0000785cm

$\frac{1}{0.0000785\text{cm}} = 12738.9\ \text{cm}^{-1}$

Wavelength of 400 cm^{-1} Raman shift in nm

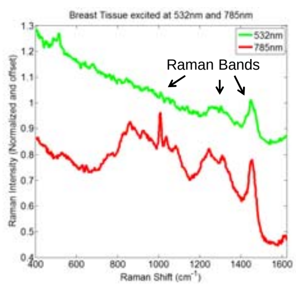
$12738.9\ \text{cm}^{-1} - 400\ \text{cm}^{-1} = 12338.9\ \text{cm}^{-1}$

$\frac{1}{12338.9\ \text{cm}^{-1}} = 0.0000810\text{cm}$

810nm

***The Raman shift is independent of excitation frequency… the spectral band positions will be the same whether you excite with a 785nm laser or a 633 nm laser

Raman Spectroscopy: Wavelength Axis



532nm excitation gives a fluorescent background while 785nm does not.

Excitation Frequency

785nm = 0.0000785cm
532nm = 0.0000633cm

$\frac{1}{0.0000785\text{cm}} = 12738.9\ \text{cm}^{-1}$

$\frac{1}{0.0000532\text{cm}} = 18797.0\ \text{cm}^{-1}$

Wavelength of 400 cm^{-1} Raman shift in nm

$12738.9\ \text{cm}^{-1} - 400\ \text{cm}^{-1} = 12338.9\ \text{cm}^{-1}$

$\frac{1}{12338.9\ \text{cm}^{-1}} = 0.0000810\text{cm}$

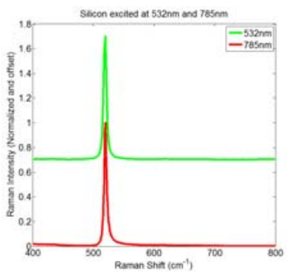
810nm

$18797.0\text{cm}^{-1} - 400\ \text{cm}^{-1} = 18397.0\text{cm}^{-1}$

$\frac{1}{1/18797.0\ \text{cm}^{-1}} = 0.0000649\text{cm}$

543nm

Raman Spectroscopy: Wavelength Axis



Silicon is not fluorescent, so you get pretty much the same Raman signal.

Excitation Frequency

785nm = 0.0000785cm
532nm = 0.0000633cm

$$\frac{1}{0.0000785\text{cm}} = \mathbf{12738.9\ cm^{-1}}$$

$$\frac{1}{0.0000532\text{cm}} = \mathbf{18797.0\ cm^{-1}}$$

Wavelength of 400 cm^{-1} Raman shift in nm

$12738.9\ cm^{-1} - 400\ cm^{-1} = 12338.9\ cm^{-1}$

$$\frac{1}{12338.9\ cm^{-1}} = 0.0000810\text{cm}$$

810nm

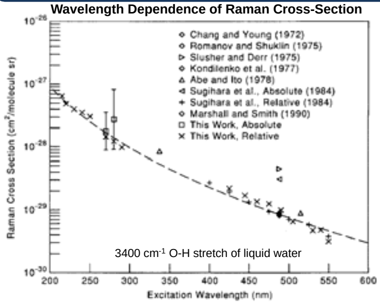
$18797.0cm^{-1} - 400\ cm^{-1} = 18397.0cm^{-1}$

$$\frac{1}{1/18797.0\ cm^{-1}} = 0.0000649\text{cm}$$

543nm

Raman Spectroscopy: Excitation Choice

Wavelength Dependence of Raman Cross-Section

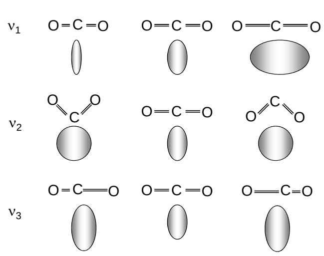


Important advantage of Raman… wavelength select ability to avoid light absorption and fluorescence… **however** the higher the wavelength the lower the Raman efficiency (Raman Cross Section).

Appl. Opt. **36**, 2686-2688 (1997)

Raman Spectroscopy: Selection Rules

For a vibration to be Raman active the incident electromagnetic field must cause a change in polarization

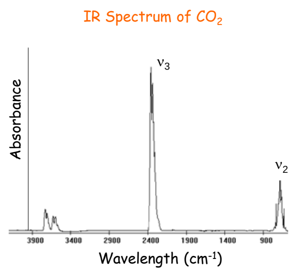


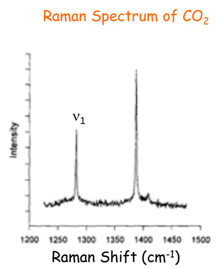
ν_1: Not IR Active; Raman Active

ν_2: IR Active; Not Raman Active

ν_3: IR Active; Not Raman Active

Raman Spectroscopy: Selection Rules

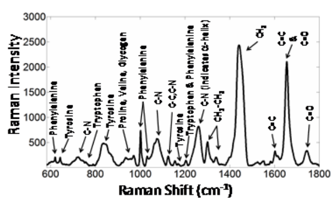




http://science.widener.edu/svb/ftir/ir_co2.html

Physics Letters A, 227 pg 237-240 (1997)

Raman Spectroscopy: Information

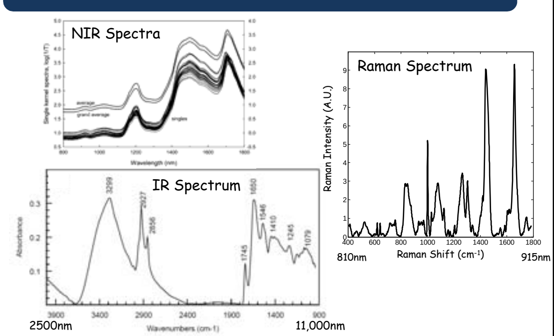


Raman Bands result from the Vibrations of specific chemical Bonds.

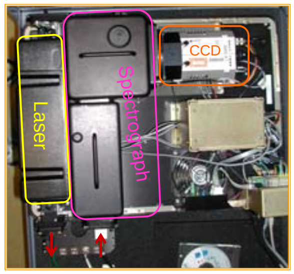
Soybean band assignments:

Wave number	Molecule	Assignment
800-900	C-C	Backbone
1003	Phe	Ring breathing
1010-1200	C-C	Backbone
1265	RC=ONH$_2$	Amide III
1450	CH$_2$	Stretch
1660	RCH=CHR	Cis
1670	RCH=CHR	Trans
1750	RC=OOR	Ester

Different types of spectroscopy



Raman Spectroscopy: Instrumentation



Kaiser Optical

Raman Spectroscopy: Instrumentation

Laser

-Generally Continuous Wave multimode lasers are used

-Narrow Spectral band (laser line filters)

-Temperature stability (electrical stability)

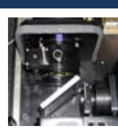
-Fiber coupling is common

-Laser power depends on wavelength and the application
-(5-100mW is common)

Kaiser Optical

Raman Spectroscopy: Instrumentation

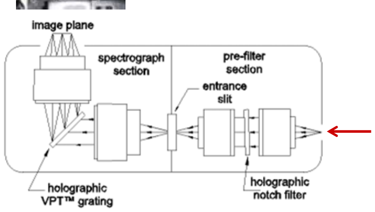
Spectrograph



-This is a axial dispersive spectrograph

-Reflective spectrographs are also common

-Interferometers are used for FT-Raman



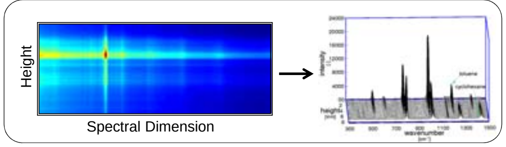
Kaiser Optical

Raman Spectroscopy: Instrumentation

Detector

-Charged-coupled devices (CCDs) are the most common detectors (256x1024 pixel array)

-FT Raman is used for frequencies where CCDs have low quantum efficiency



Advantages

Advantages:

-Little sample preparation (Polishing and fixing to a slide is common)

-Not sensitive to water (Good for biological samples)

-High chemical specificity (Narrow spectral bands)

-Qualitative and Quantitative information

-Non-destructive (A measurement does not chemically or physically change the sample)

-Can take measurements on solids, liquids, or gases

-Measurements are taken without touching the sample (Remote sensing)

-Easily coupled with fiber-optics

Disadvantages

Disadvantages

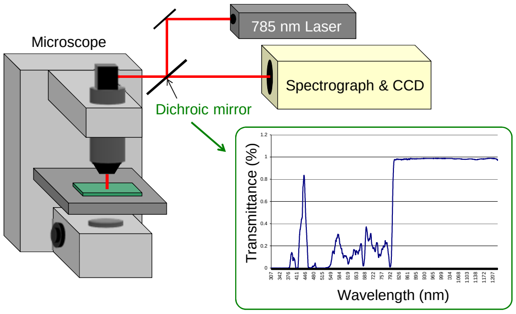
-Acquisition times tend to be longer than other techniques (real time measurements have been demonstrated… but something like video rate imaging is not yet a reality)

-Raman signal tends to be weak

-Raman signal is often mixed with a fluorescent background signal, which can make signal processing and quantification difficult.

-High laser powers can burn delicate samples

Coupling Raman to a Microscope



Raman Microscopy Example

Kinetic Experiments to monitor reactions
(bonds forming and/or breaking)

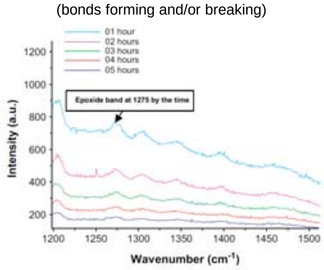
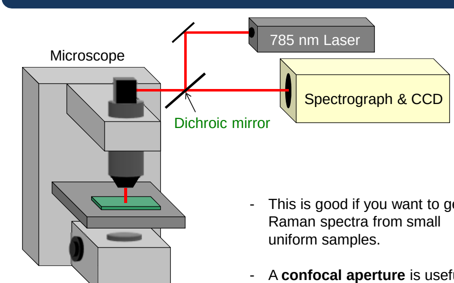


Fig. 4. Raman spectra for RTM6 at 150°C.

Polymer Testing 28 (2009) 42-45

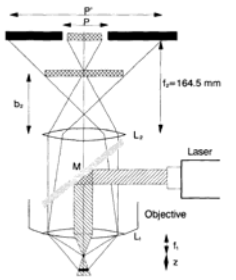
Coupling Raman to a Microscope



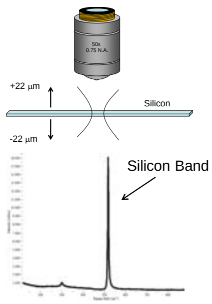
- This is good if you want to get Raman spectra from small uniform samples.
- A **confocal aperture** is useful for improving spatial and Depth resolution

Confocal Raman Spectroscopy

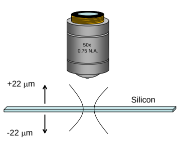
Tabaskblat et al., Appl. Spec 1992

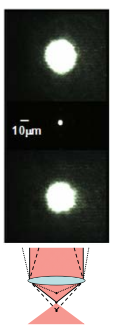


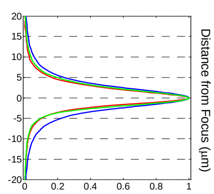
The Experiment



Confocal Raman Spectroscopy

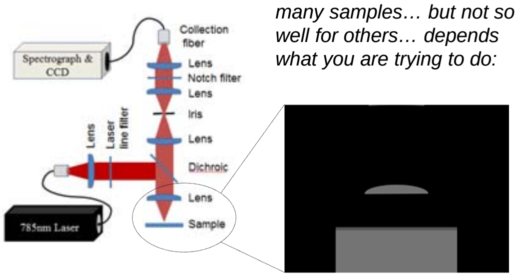






Conventional Raman Spectroscopy

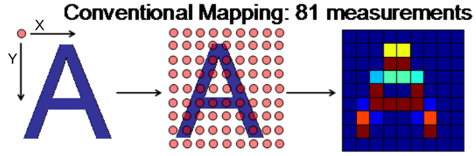
Works really well for many, many samples… but not so well for others… depends what you are trying to do:



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

Raman Imaging

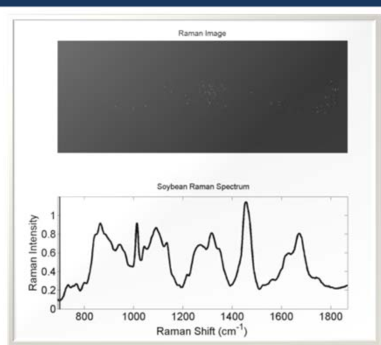
Conventional Mapping: 81 measurements



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

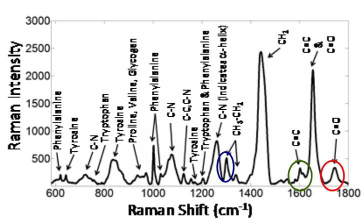
****There are other ways to do Raman Imaging, but this is by far the most common*

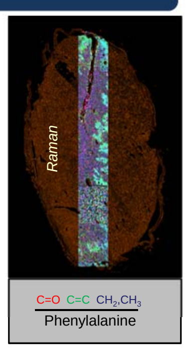
Chemical Distribution by Raman



***This is a soybean section cut by microtome to 5μm thick

Chemical Distribution

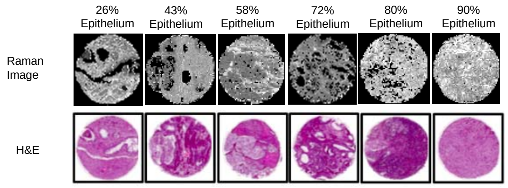




***Serial soybean sections cut by microtome to 5μm thick

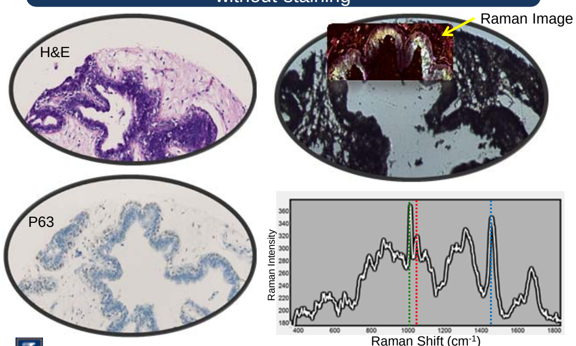
Raman Images of Prostate Biopsies

***Another Example of Raman Imaging**:*



M. Schulmerich, R. Bhargava, et. al. J. Biomed. Opt.- *In Preparation*

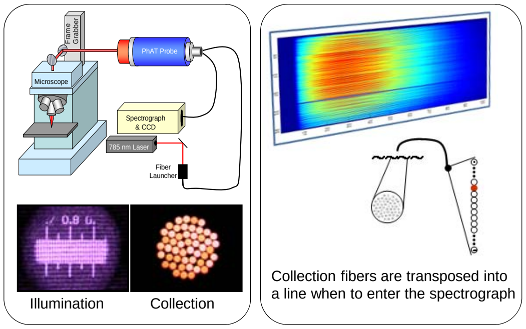
Raman can give you some histological information without staining



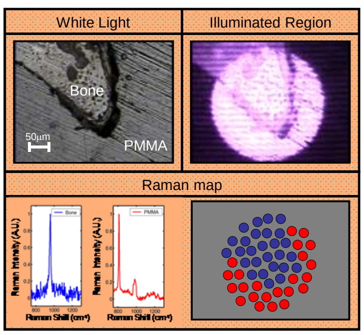
Raman Imaging

- Raman Imaging by point mapping is by far the most common approach and is what most commercial Instruments offer
- Next I'm going to highlight a few other Raman imaging methods that have been used… but keep in mind that these are pretty uncommon
  - Hyper spectral Mapping
  - Line scan imaging
  - Wide Field imaging

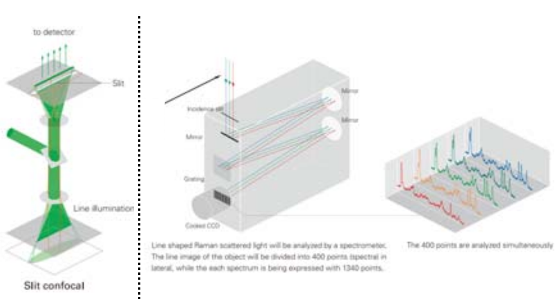
Hyperspectral Raman mapping



Hyperspectral Raman mapping

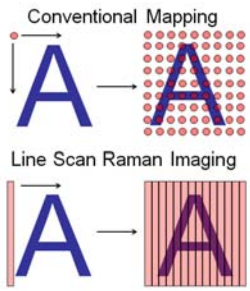


Line Scan Raman mapping



www.nanophotn.jp

Line Scan Raman mapping

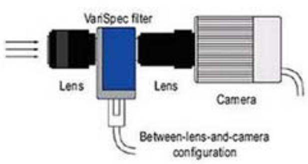


The major benefit is time to collect an image

Images generally look less pixelated

The ability to reject out of focus light can be limited depending on how the line scanning is implemented

Wide-field Imaging



Tunable filter can replace the spectrograph to collect wide-field images.

The filter is swept through a range of wavelengths and the frames are stacked to obtain spectra.

http://www.channelsystems.ca

Comparison

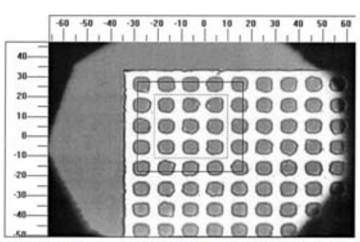


Figure 1. Bright-field microscope image of the examined SEM standard sample with a micrometer scale delineated at the top and left. The dark squares (edge length, 5 μm) corresponds to the underlying Si substrate. The area covered in the Raman mapping experiments is indicated by the larger square (45 μm × 45 μm, solid lines). The smaller square (~30 μm × 30 μm, dotted lines) represents the area for which the Raman images obtained by each methodology are presented in Figures 2–4.

Levin et. al. Anal. Chem. 2003, 75, 4312-4318

Point Mapping

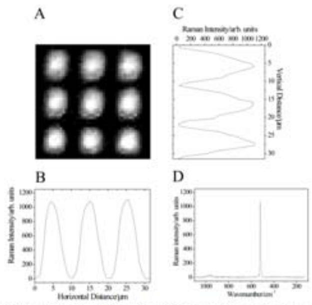


Figure 2. Raman image (A) at 520 cm^{-1} reconstructed from point mapping data. The horizontal (B) and vertical (C) intensity profiles along a central pixel of the middle Si square and the corresponding Raman spectrum (D) are shown.

Levin et. al. Anal. Chem. 2003, 75, 4312-4318

Line Scanning

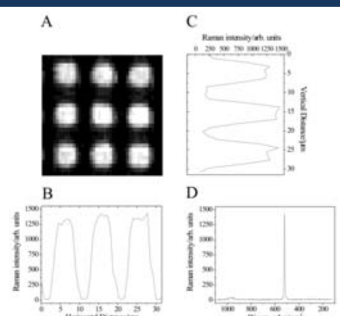


Figure 3. Raman image (A) at 520 cm^{-1} reconstructed from line mapping data. The horizontal (B) and vertical (C) intensity profiles along a central pixel of the middle Si square and the corresponding Raman spectrum (D) are shown.

Levin et. al. Anal. Chem. 2003, 75, 4312-4318

Wide-Field Imaging

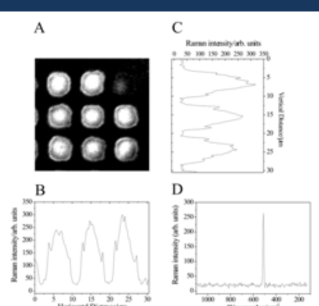


Figure 4. Global Raman image (A) obtained with the LCTF band-pass centered at 520 cm^{-1}. The horizontal (B) and vertical (C) intensity profiles along the central pixel of the middle Si square and the corresponding Raman spectrum (D) are shown. The global Raman image (E) was obtained with a 30 times longer exposure time of 5 min compared to image (A).

Levin et. al. Anal. Chem. 2003, 75, 4312-4318

Imaging Comparison

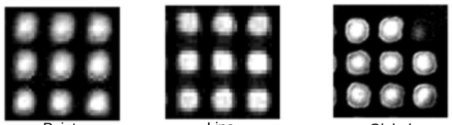


Table 1. Parameters Employed for Point and Line Mapping and Global Raman Imaging Experiments*

	point mapping		line mapping		global imaging
	sample limited	laser limited	sample limited	laser limited	sample limited
power density (MW/cm²)	0.008	13.5	0.008	0.096	0.008
spectral resolution	4 cm⁻¹		4 cm⁻¹		
spectral band-pass					7 cm⁻¹ (fwhm)
spatial resolution	1.1 μm		1.1 μm (horizontal) 1.06 μm (vertical)		0.313 μm
signal-to-noise ratio	29	9	77	40	14
total acquisition time	11 h 24 min	1h 16 min	12 min 34 s	3 min 47 s	46 min 22 s
exposure time only	(61 × 61)(10) s = 10 h 20 min	(61 × 61)(0.1) s ≈ 6 min 12 s	61(10) s = 10 min 10 s	61(1) s = 1min 1 s	194(10) s = 32 min 20 s

* The sample limited cases are used forcomparison of all three methodologies.

Levin et. al. Anal. Chem. 2003, 75, 4312-4318

Disadvantages

Again…. The Disadvantages

-Acquisition times tend to be longer than other techniques (real time measurements have been demonstrated… but something like video rate imaging is not yet a reality)

-Raman signal tends to be weak

-Raman signal is often mixed with a fluorescent background signal, which can make signal processing and quantification difficult.

-High laser powers can burn delicate samples

Various technique address some of these disadvantages

Other Raman Techniques

-Surfaced enhanced Raman spectroscopy (SERS)

-Tip enhanced Raman spectroscopy (TERS)

-Resonance Raman spectroscopy

-Raman tomography

-Coherent anti-stokes Raman spectroscopy (CARS)

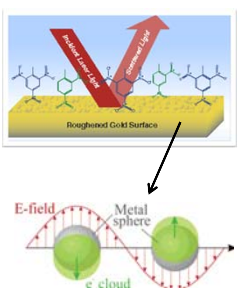
-Stimulated Raman spectroscopy

-Others I can't think of at the moment and much more to come!

Thank you

Questions?

Surfaced Enhance Raman Spectroscopy



- The Raman scatter can be enhanced by 10 to 11 orders of magnitude with SERS… can detect single molecules
- Mechanism: surface plasmons
- Excitation Frequency matters
- Different selection rules, so the SERs spectrum is different than the spontaneous Raman spectrum
- Reproducibility tends to be poor, and quantification with SERS is difficult, though research advances are making headway.

Schatz et al. *J. Phys. Chem. B*, **2003,** 107, 668-677