Raman Spectroscopy

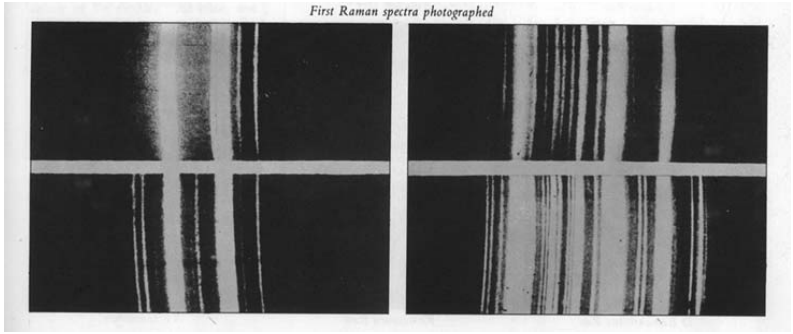
Introduction

- **Professor Sir C.V. Raman**

A New Type of Secondary Radiation
C. V. Raman and K. S. Krishnan,
Nature, 121(3048): **501-502, March 31, 1928**

The Nobel Prize in Physics 1930
"for his work on the scattering of light and for the discovery of the effect named after him"

1888-1970

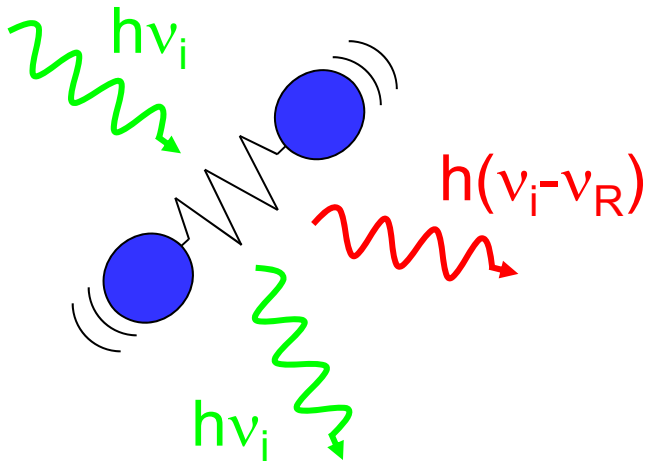


First photographed Raman spectra

Bangalore, India

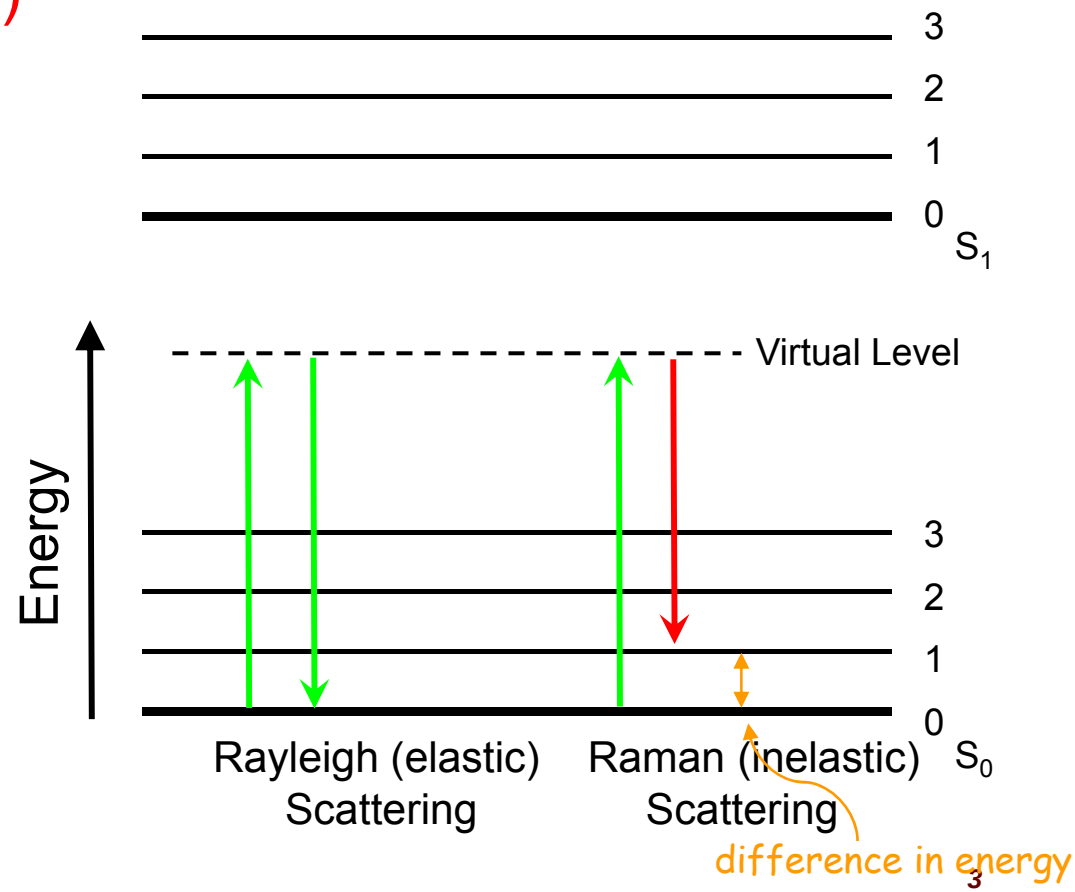
Introduction

- **The Raman Effect → Inelastic Scattering**



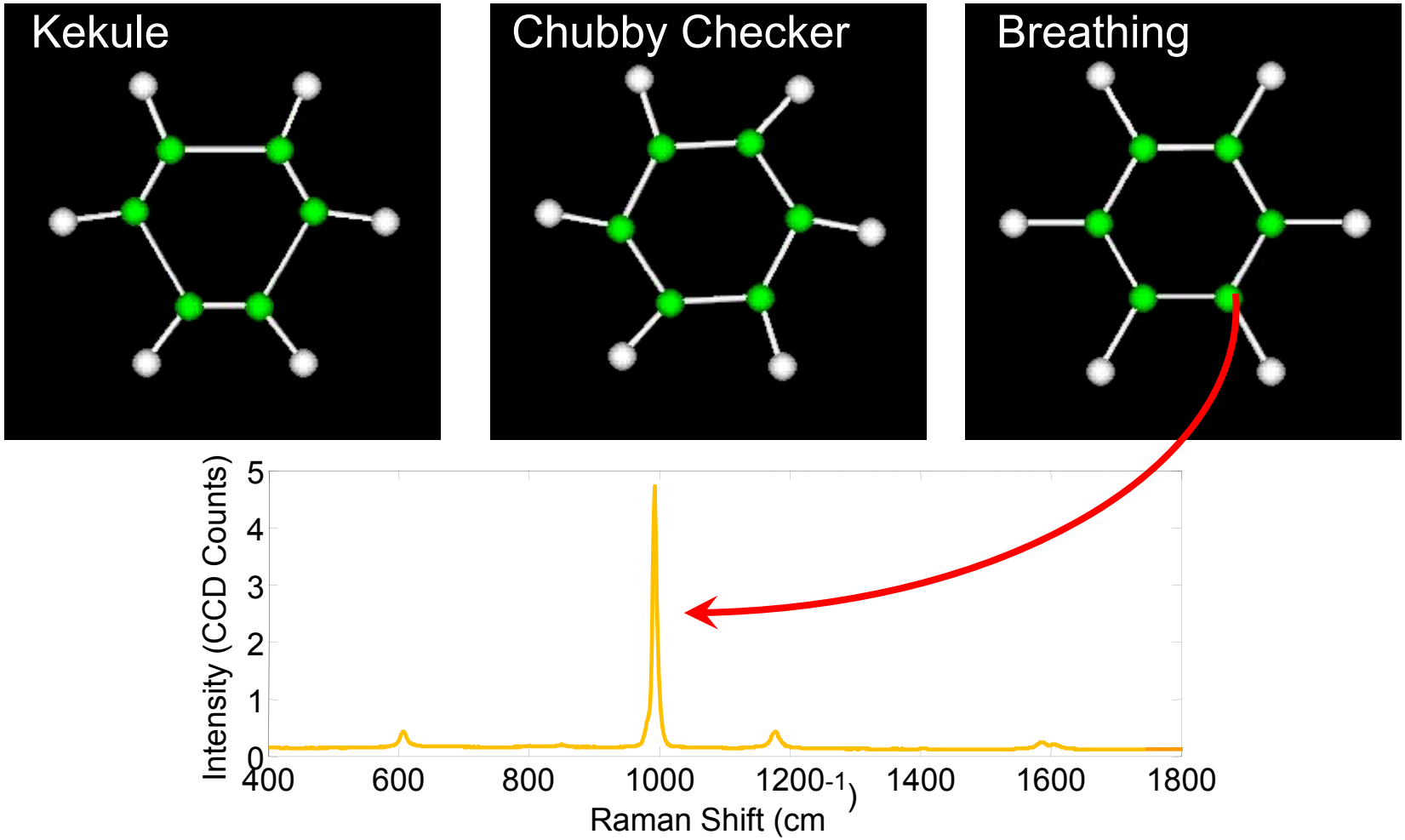
Inelastic Scattering

- Energy transferred from incident light to molecular vibrations
- Emitted light has decreased energy ($\lambda_i < \lambda_R$)



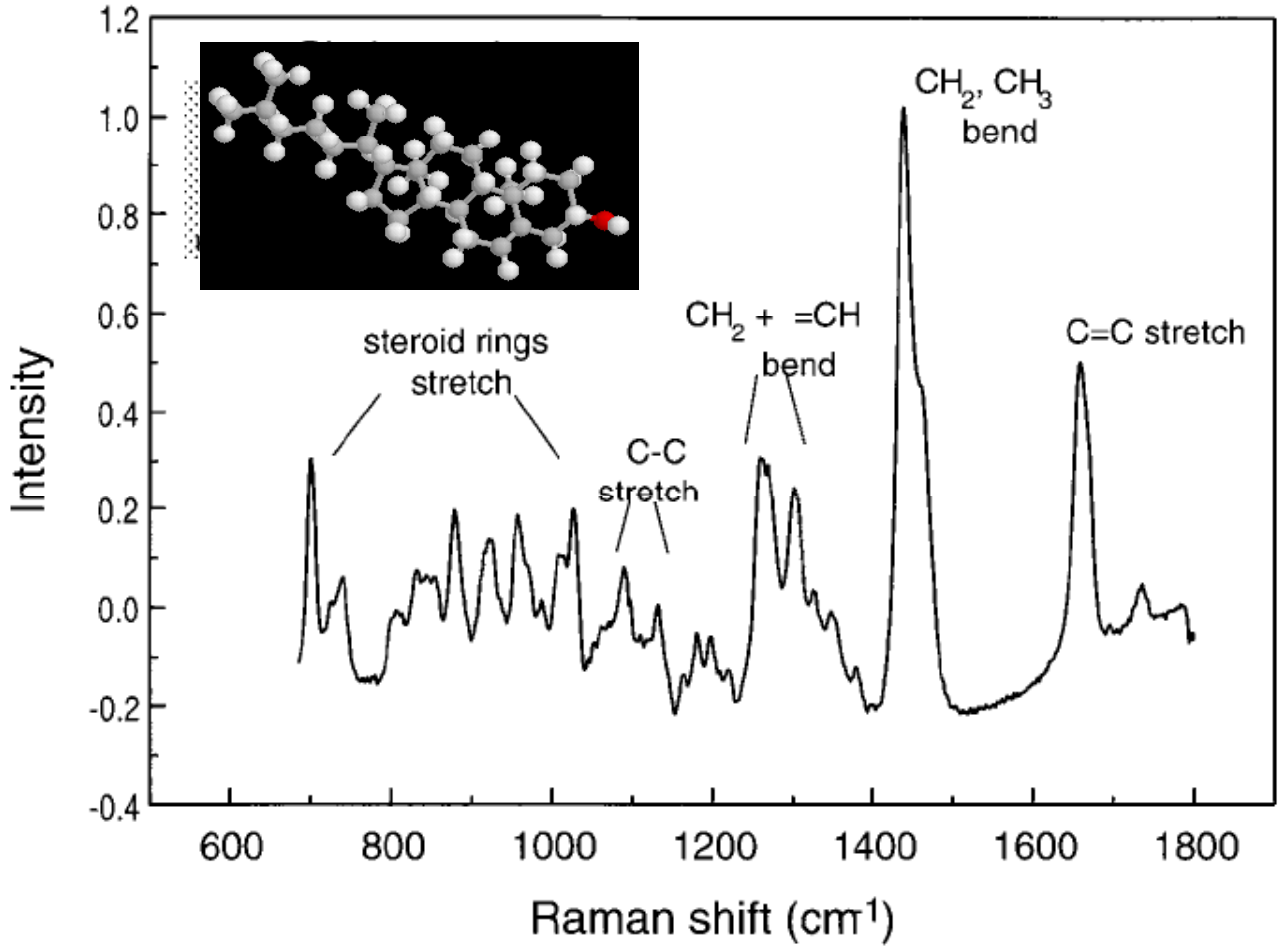
Introduction

- **Some Vibrations in Benzene**



Introduction

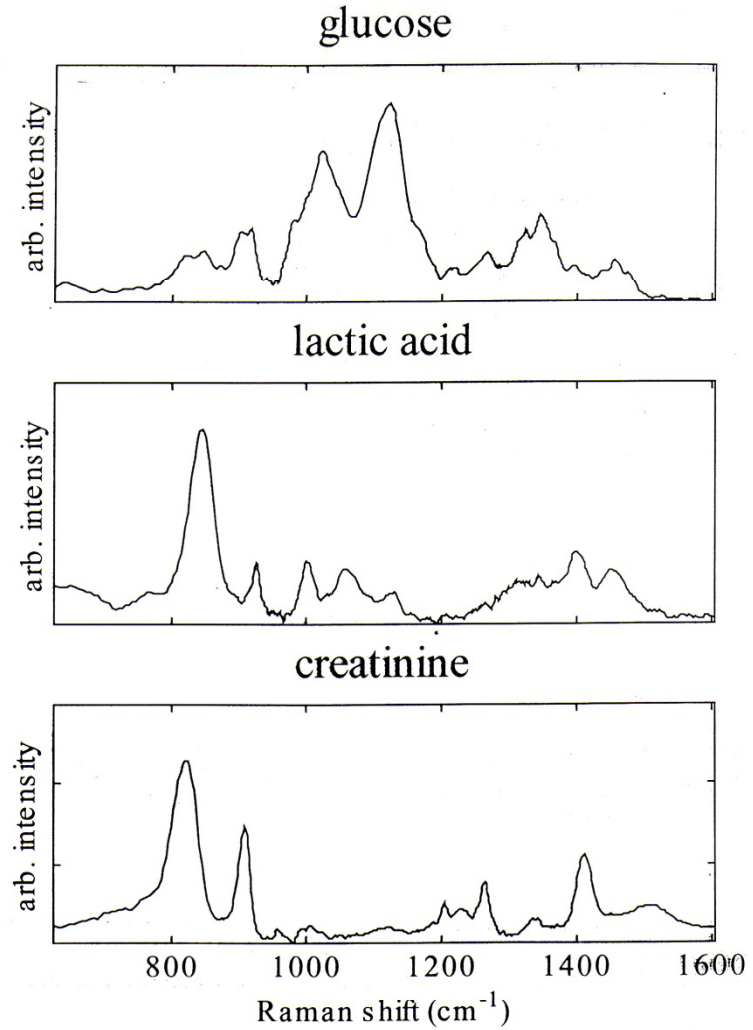
- **Raman Spectrum of Cholesterol**



Hanlon *et al.* "Prospects for *in vivo* Raman spectroscopy," Phys Med Biol **45**: R1 (2000)

Introduction

- **Raman Spectra → Fingerprinting a Molecule**
  - Raman spectra are molecule specific
  - Spectra contain information about vibrational modes of the molecule
  - Spectra have sharp features, allowing identification of the molecule by its spectrum



Examples of analytes found in blood which are quantifiable with Raman spectroscopy

Introduction

Evolution of Raman Spectroscopy

- **1928~1960**
  - Minor experimental advances
- **1960**
  - Invention of laser expands scope experiments
- **1980s: rapid technological advances**
  - Fourier Transform spectroscopy
  - Charge Coupled Device (CCD) detectors
  - Holographic and dielectric filters
  - Near-Infrared (NIR) lasers
- **Late 1980s→1990s**
  - Biomedical investigations
  - Advanced dispersive spectrometers
- **2000 →**
  - In vivo application
  - Optical fiber probes
  - Non-linear spectroscopy

Introduction

- **Applications of Raman Spectroscopy**
  - Structural chemistry
  - Solid state
  - Analytical chemistry
  - Applied materials analysis
  - Process control
  - Microspectroscopy/imaging
  - Environmental monitoring
  - Biomedical

Introduction

History of Biological Raman Spectroscopy

- **1970: Lord and Yu record 1st protein spectrum from lysozyme using HeNe excitation**
- **Evolution to NIR excitation**
  - Decreased fluorescence, Increased penetration (mm)
- **1980s:**
  - FT Raman with Nd:YAG and cooled InGaAs detectors (long collection times (30 min))
  - Clarke (1987-1988): visible excitation of arterial calcium hydroxyapatite and carotenoids
- **1990s, advances in:**
  - Lasers, Detectors, Dispersive spectrometers, Filters
  - Chemometrics

Introduction

- **Diagnostic Advantages of Raman Spectroscopy**
  - Wavelength selection (from UV to IR)
  - No biopsy required
  - Directly measures molecules
    - Small concentrations
    - Chemical composition
    - Morphological analysis
  - Quantitative analysis
  - In vivo diagnosis

Classical Raman Physics

- **Interaction between electric field of incident photon and molecule**
  - Electric field oscillating with incident frequency f_i:

$$E_i = E_0 \cos(2\pi f_i t)$$

  - Induces molecular electric dipole (p):

$$\vec{p} = \alpha \vec{E}$$

    - Proportional to molecular polarizability, α
      - ease with which the electron cloud around a molecule can be distorted
  - Polarization results in nuclear displacement

$$q = q_0 \cos\left(2\pi \nu_R t\right)$$

Classical Raman Physics

- For small distortions, polarizability is linearly proportional to the displacement

$$\alpha = \alpha_0 + \left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 + \ldots$$

- Resultant dipole:

Rayleigh Scattering

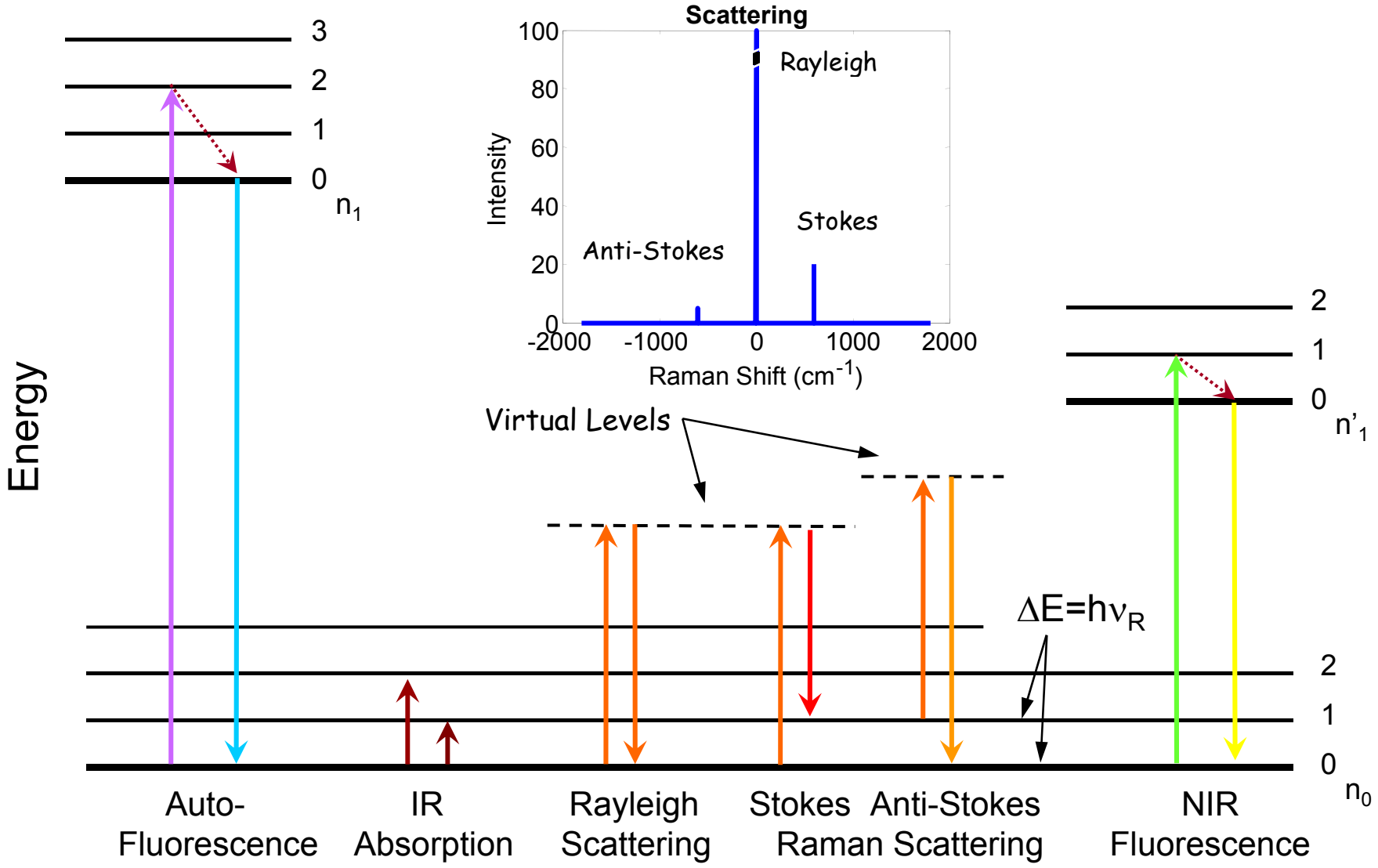
$$\vec{p} = \alpha \vec{E} = \alpha_0 E_0 \cos\left(2\pi \nu_i t\right) +$$

$$\frac{1}{2} E_0 q_0 \left(\frac{\partial \alpha}{\partial q}\right)_0 \left\{\cos\left[2\pi\left(\nu_i + \nu_R\right)t\right] + \cos\left[2\pi\left(\nu_i - \nu_R\right)t\right]\right\}$$

Anti-Stokes Raman

Stokes Raman

Photo-Molecular Interactions



Classical Raman Physics

- **Raman scattering occurs only when the molecule is 'polarizable'**

$$\frac{\partial \alpha}{dq} \neq 0$$

- **Raman intensity $\propto f^4$**
  - Classical dipole radiation
  - Stokes shifted Raman is more intense than anti-Stokes by Boltzmann factor:

$$\frac{I_A}{I_S} = \left(\frac{f_i + f_R}{f_i - f_R} \right)^4 e^{-\frac{hf_R}{kT}}$$

- **Consistent with other scattering phenomena, often reported in terms of cross-section (σ [cm^2]), or probability of scattering:**

$$I = I_0 \sigma \rho l$$

  - ρ: density of molecules
  - l: pathlength

Characteristics of Raman Scattering

- **Very weak effect**
  - Only **1 in 10^7** photons is Raman scattered
  - NIR elastic scattering in tissue: $1/\mu_s' \approx 1mm$
  - NIR absorption in tissue: $1/\mu_a \approx 10cm$
  - Red absorption in tissue or water: $1/\mu_a \approx 5m$
  - Raman scattering in tissue or water: $1/\mu_R \approx 3km$
- **True scattering process**
  - Virtual state is a short-lived distortion of the electron cloud which creates molecular vibrations
  - $\tau < 10^{-14}$ s
- **Strong Raman scatterers have distributed electron clouds**
  - C=C
  - π-bonds

Units & Dimensional Analysis

- **Spectroscopic frequencies reported in wavenumbers [cm^{-1}], proportional to transition energy :**

$$\tilde{\nu} = \frac{E}{hc} = \frac{f}{c} = \frac{1}{\lambda} \qquad \boxed{\begin{aligned} E &= hf \\ c &= \lambda f \end{aligned}}$$

- **Raman frequencies are independent of excitation wavelength and reported as shifts**
  - Wavenumbers relative to excitation frequency:

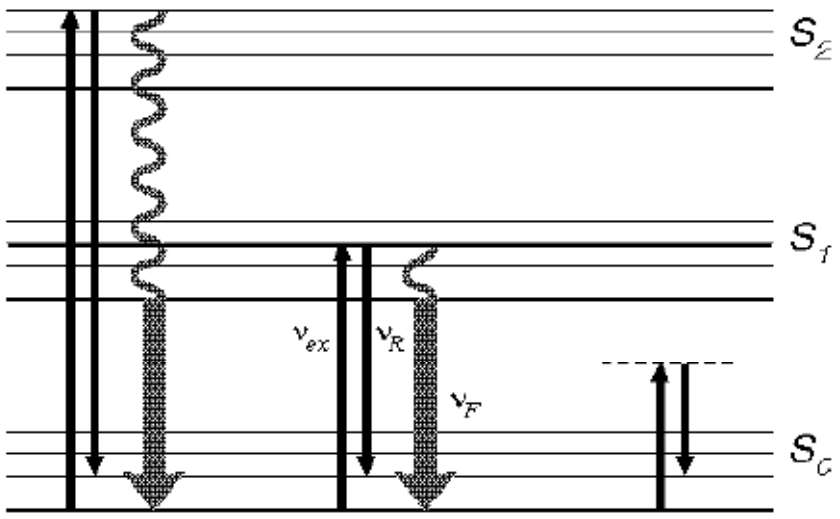
$$\tilde{\nu}_R = \frac{1}{\lambda_i} - \frac{1}{\lambda_R}$$

Units & Dimensional Analysis

- **Example**
  - NIR excitation at 830 nm: 12,048 cm^{-1}
  - Typical Raman shift: ~1000 cm^{-1}
    - λ_R = 905 nm
  - Sharp biological Raman linewidths ~10 cm^{-1} FWHM
    - $\Delta\lambda_R$= 0.69 nm

UV, Visible, and NIR Excitation

- **Wavelength Selection**
  - Raman signals have a constant shift → can vary excitation wavelength and get same information
- **UV**
  - **+ Resonance enhanced**
  - **+ $\lambda_R < \lambda_F$ → filter fluorescence**
  - **- photo damage, low penetration**
- **Visible**
  - **+ Raman $\propto \lambda^{-4}$ → $\uparrow I_R$ vs. IR**
  - **- fluorescence overlaps with Raman signal**
- **NIR:**
  - **+ Low fluorescence**
  - **+ Deep penetration**
  - **- Raman $\propto \lambda$-4 → $\downarrow$IR vs. Vis**



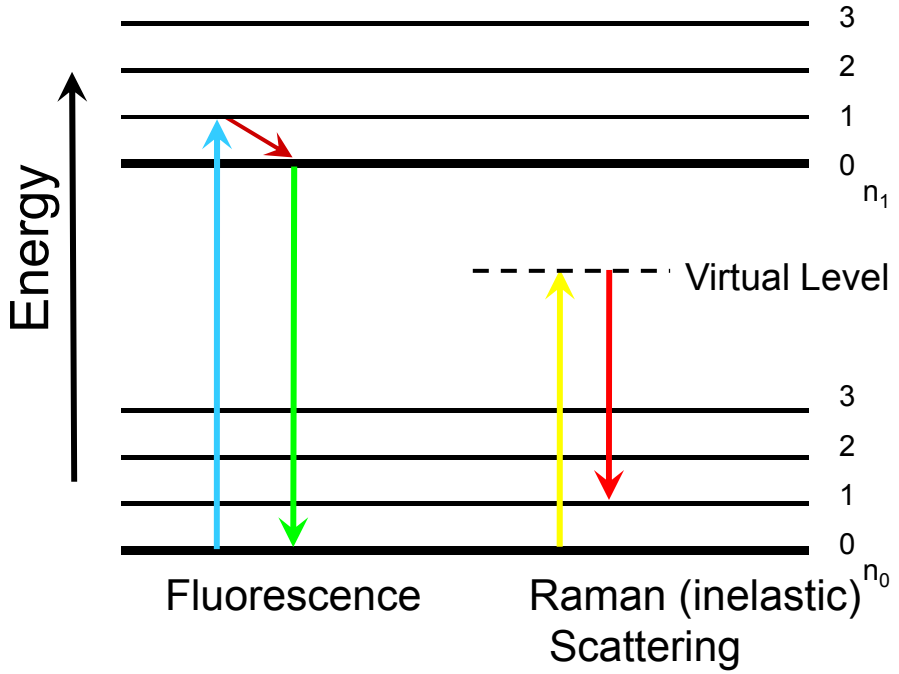
UV, Visible, and NIR Excitation

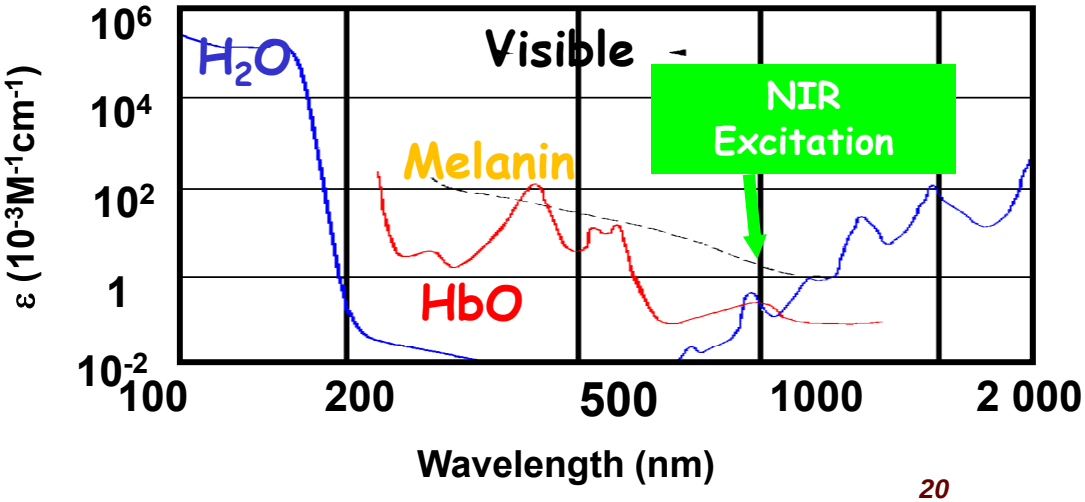
Applications

- **UVRR**
  - Biological macromolecules: nucleic acids, proteins, lipids
  - Organelles, cells, micro-organisms, bacteria, phytoplankton neurotoxins, viruses
  - Clinically limited: photomutagenicity
- **Visible**
  - Cells (minimal fluorescence)
  - DNA in chromosomes, pigment in granulocytes and lymphocytes, RBCs, hepatocytes
  - First artery studies: hydroxyapatite and carotenoids (Clarke 1987, 1988)
- **NIR**
  - Hirschfeld & Chase, 1986: FT-Raman
  - Tissue: artery, cervix, skin, breast, blood, GI, esophagus, brain tumor, Alzheimer's, prostate, bone

UV, Visible, and NIR Excitation

- **Spectroscopic Advantages of NIR Raman**
  - Narrow vibrational bands are chemical specific and rich in information
  - Freedom to choose excitation wavelength
  - Minimize unwanted tissue fluorescence
  - optimize sampling depth
  - Utilize CCD technology

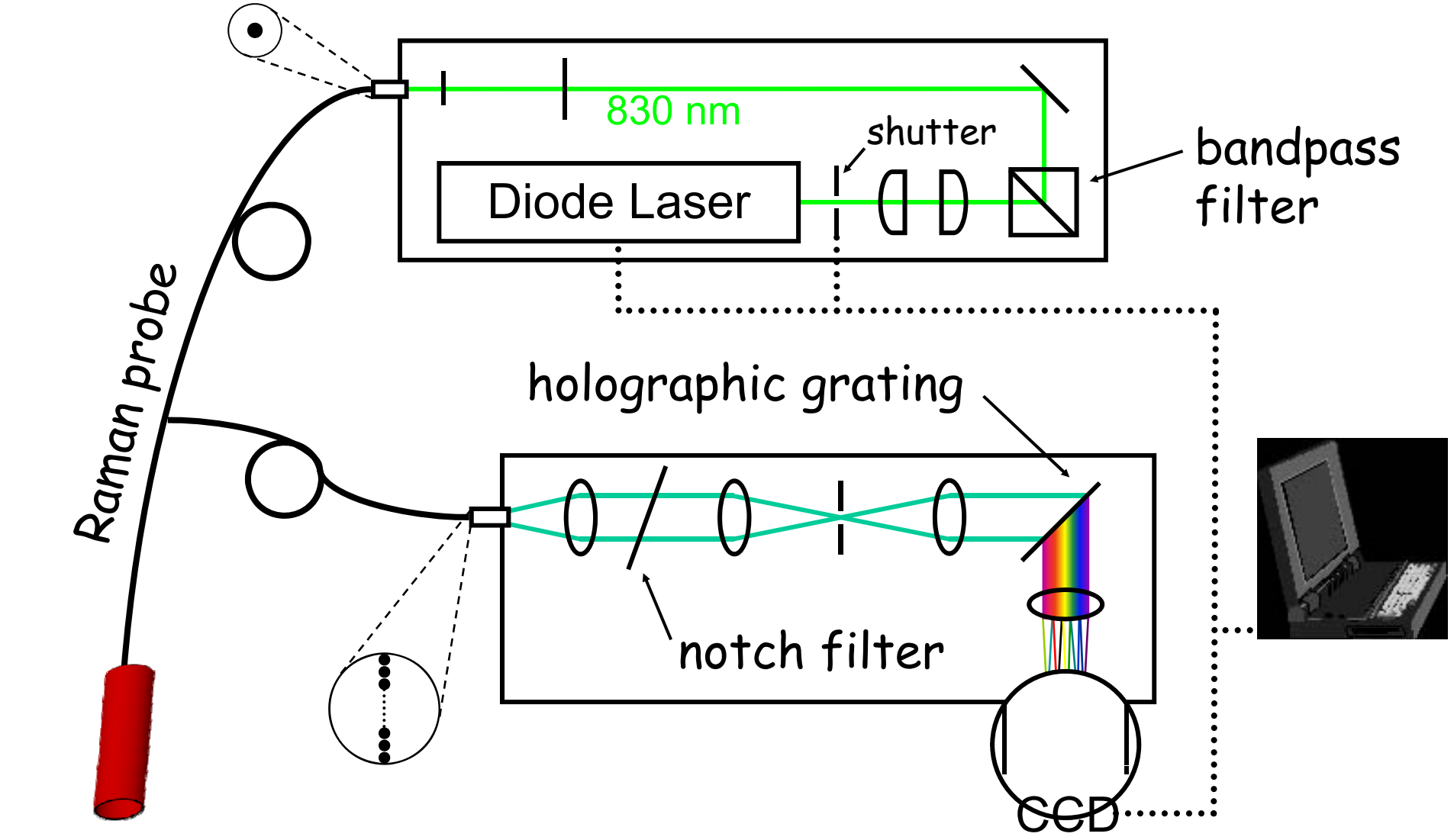




Current Raman Instrumentation

- **Laser diodes**
  - Compact, Stable narrow line, NIR
- **High throughput spectrographs (f/1.8)**
- **Holographic elements**
  - Bandpass filters (eliminates spontaneous emission of lasing medium)
  - Notch filters (10^6 rejection of Rayleigh scattered laser line)
  - Large area, highly efficient transmission gratings
- **CCD detectors**
  - High QE (back-thinned, deep-depletion)
  - Low noise (LN2 cooled)
  - Multichannel detection
- **High throughput, filtered fiber optics probes**
- **NIR FT and scanning PMT systems no longer useful**

Clinical Raman Systems

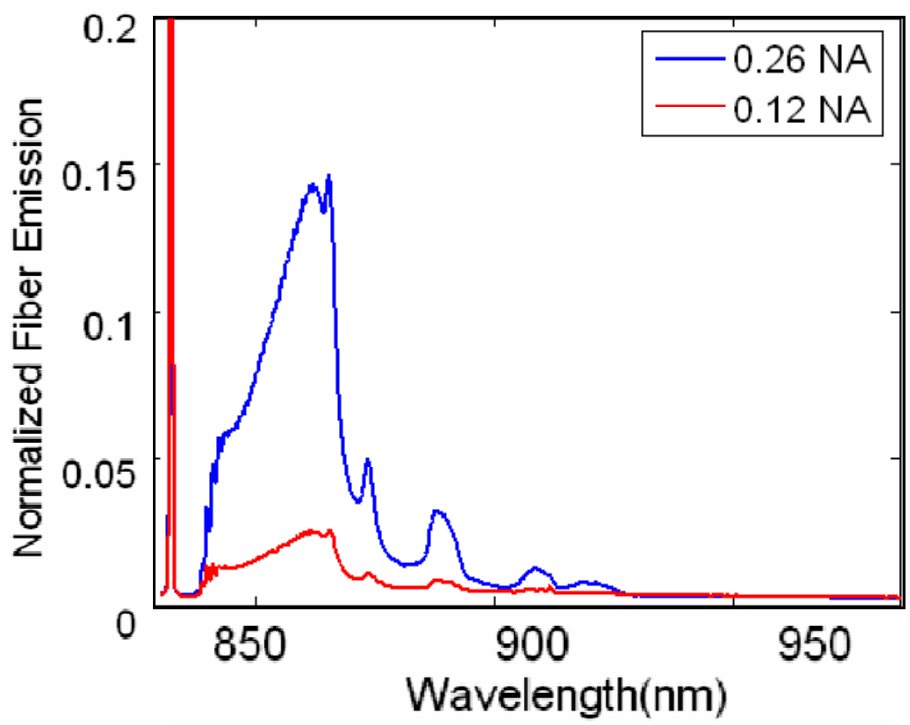


Fiber Raman Probe Design

Problems

- **Fiber background**
  - Distorts signal
  - Adds shot-noise
- **Low signal collection**
  - Raman effect is weak
  - Tissue is highly diffusive

Fiber background $\propto NA^2$



Fiber Raman Probe Design

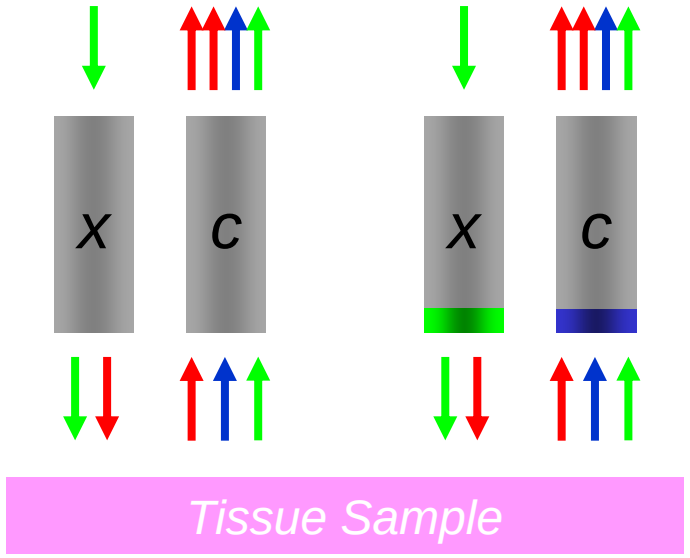
- **Reduce Fiber Background**
  - Fiber background produced equally in excitation and collection fibers
  - **Excitation laser → Raman scattered light from tissue**
  - **Fiber Raman scattering, transmitted by excitation fiber***
  - **Fiber Raman background elastically scattered from sample (and collected)**
  - **Excitation elastically scattered and gathered by collection fibers**
  - **Fiber Raman scattering by collection fibers***

* $\propto NA^2$

Excitation laser

Tissue Raman

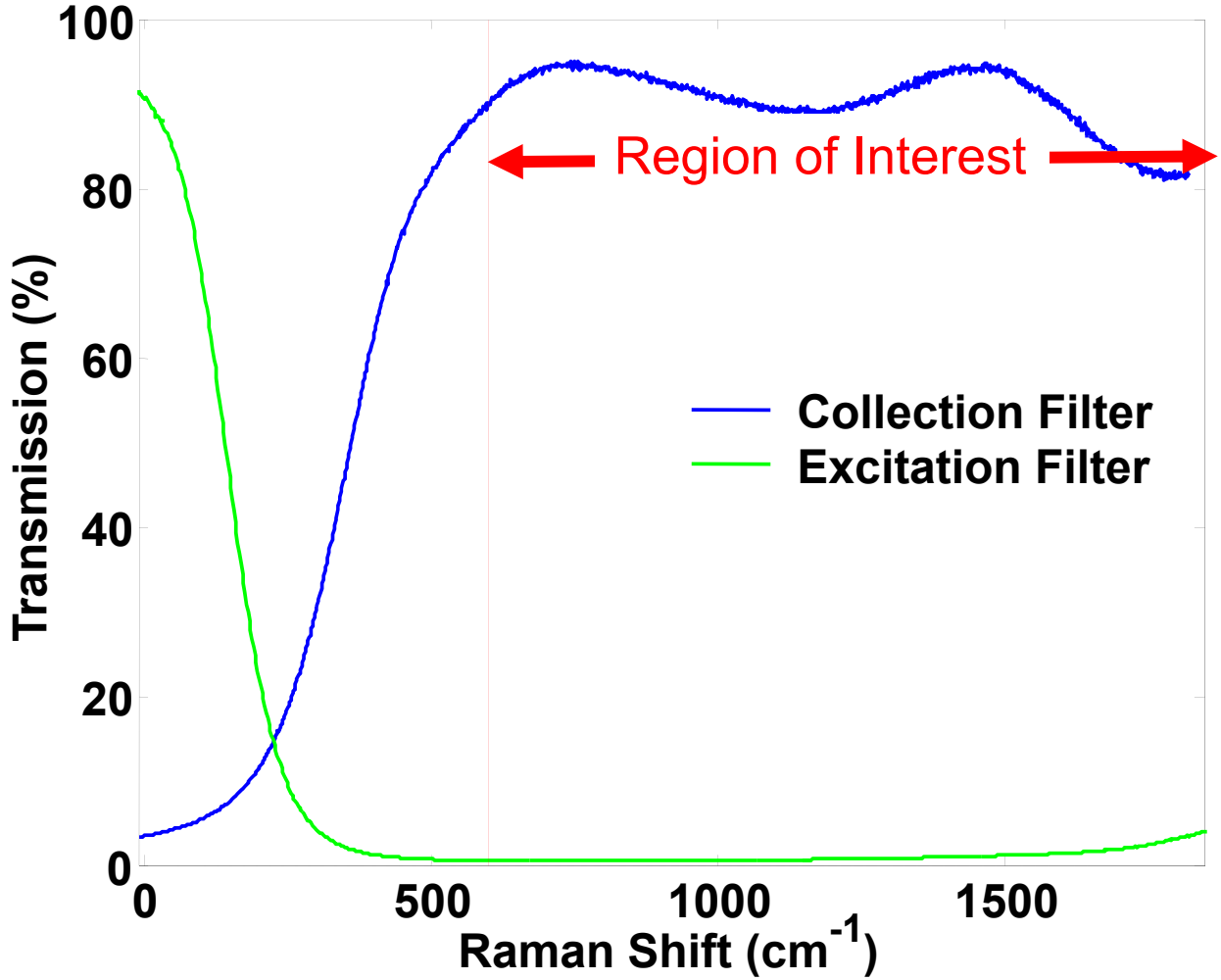
Fiber background



From McCreery RL
"Raman Spectroscopy for Chemical Analysis," 2000.

Fiber Raman Probe Design

- Filter Transmission



Fiber Raman Probe Design

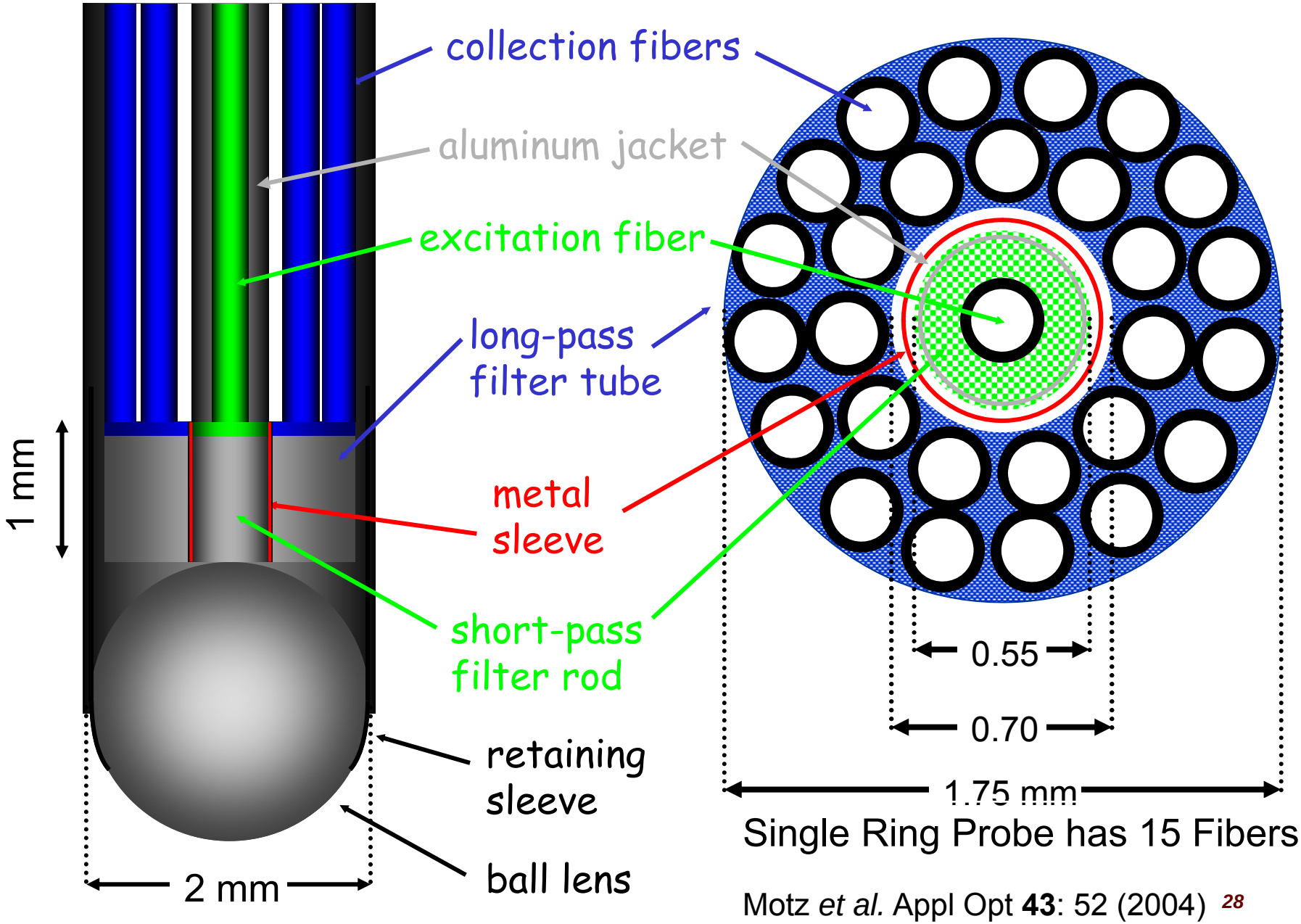
- **Problems**
- **Fiber background**
  - Distorts signal
  - Adds shot-noise
- **Low signal collection**
  - Raman effect is weak
  - Tissue is highly diffusive
- **Solutions**
- **Micro-optical filters**
  - Short-pass excitation filter
  - Long-pass collection filter
- **Optimize optical design**
  - Characterize distribution of Raman light in tissue
  - Define optimal geometry
  - Design collection optics

Fiber Raman Probe Design

Design Goals

- **Restricted geometry for clinical use**
  - Total diameter ~2mm for access to coronary arteries
  - Flexible
  - Able to withstand sterilization
- **Designed to work with 830 nm excitation**
- **High throughput**
  - Data accumulation in 1 or 2 seconds
  - Safe power levels
  - SNR similar to open-air optics laboratory system
  - Accurate application of models

Fiber Raman Probe Design



Single Ring Probe has 15 Fibers

Motz *et al.* Appl Opt **43**: 52 (2004)

Cardiovascular Disease

The Burden of Cardiovascular Disease†

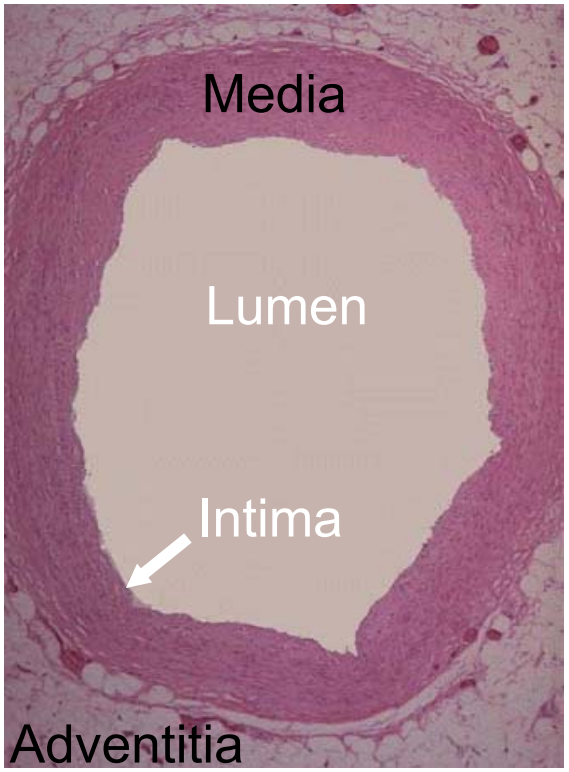
- **71,300,000 people in United States afflicted**
- **910,600 deaths per year**
  - 1 out of every 2.7 deaths
- **Coronary artery disease claims 653,000 lives annually**
  - 1 out of every 5 deaths
  - Economic cost: greater than $142.5 billion

†American Heart Association, *Heart and Stroke Statistics-2006 Update*

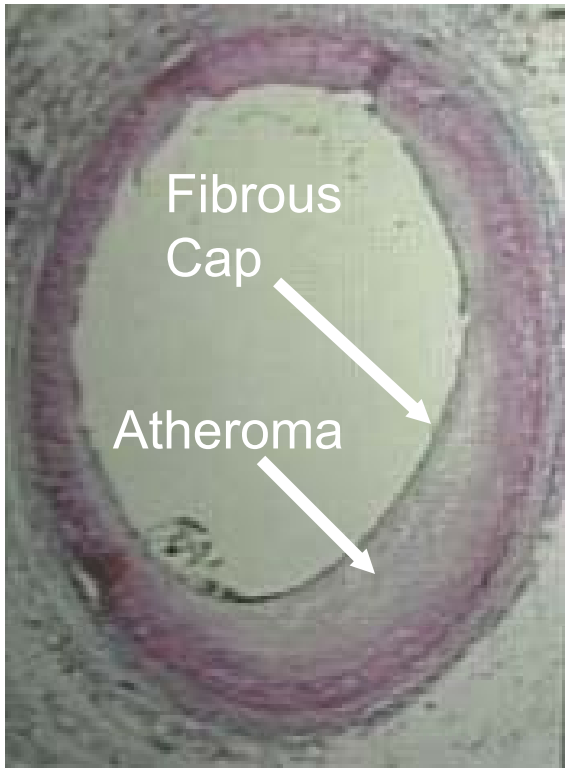
Cardiovascular Disease

- **Arterial Anatomy**

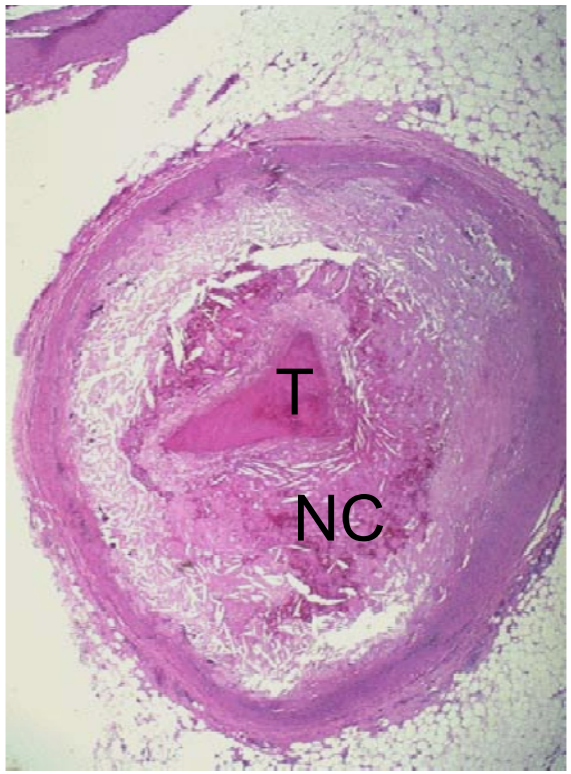
Normal



Mildly Atherosclerotic Plaque



Ruptured Plaque



T: thrombus
NC: necrotic core

Cardiovascular Disease

- **Some Current Challenges in Cardiology**
  - Evaluation and development of therapeutics
  - Etiology of atherosclerosis
  - Mechanisms of re-stenosis
    - Post-angioplasty
    - Transplant vasculopathy
  - Detection of vulnerable atherosclerotic plaques
    - Prediction/prevention of cardiac events

Cardiovascular Disease

Vulnerable Plaques

- **Account for majority of sudden cardiac death**
- **Frequently occur in clinically silent vessels**
  - <50% stenosis
- **Effective treatments unknown**
- **Characterized by:**
  - Biochemical changes
  - Foam cells
  - Lipid pool
  - Inflammatory cells
  - Thin fibrous cap (<65 μm)
- **Currently undetectable**

Cardiovascular Disease

Standard Diagnostic Techniques

- **Angiography**
  - Severity of stenosis, thrombosis, dense calcifications
  - Provides no biochemical information
- **Angioscopy**
  - Surface features of plaque, including color
  - No information of sub-surface features
- **Histopathology**
  - Biochemical and morphological information
  - Requires excision of tissue

Cardiovascular Disease

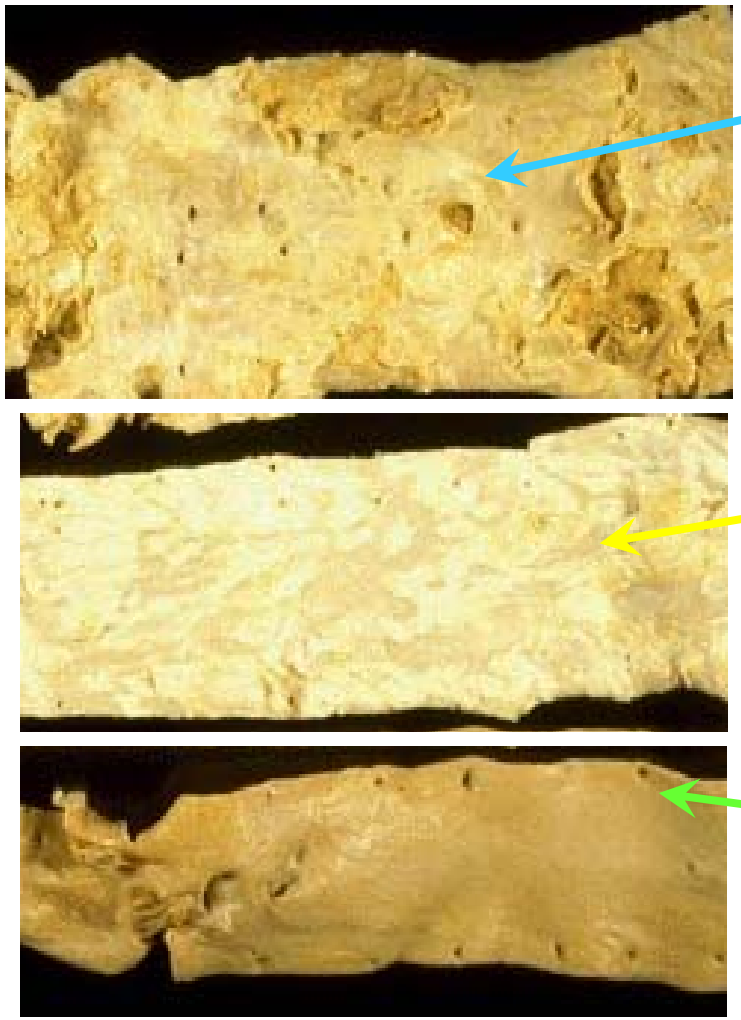
- **Emerging Diagnostic Techniques**
  - Magnetic resonance imaging
  - External ultrasound
  - Positron emission tomography
  - Electron beam computed tomography
  - Thermography
  - Elastography

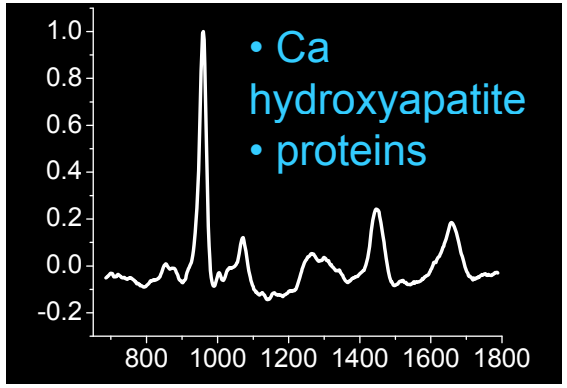
Non-Invasive

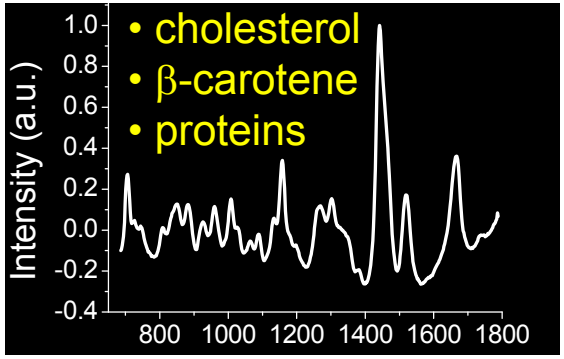
- Intravascular ultrasound
  - Microstructure (100 μm)
- Optical coherence tomography
  - Microstructure (10 μm)
- Fluorescence spectroscopy
  - Limited chemical information
  - Broad spectral features
- Raman Spectroscopy
  - Quantitative biochemical information
  - Morphological analysis

Cardiovascular Disease

- **Raman Spectral Pathology of Atherosclerosis**







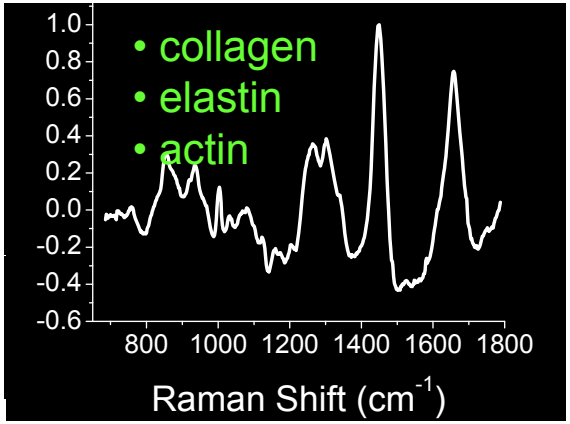
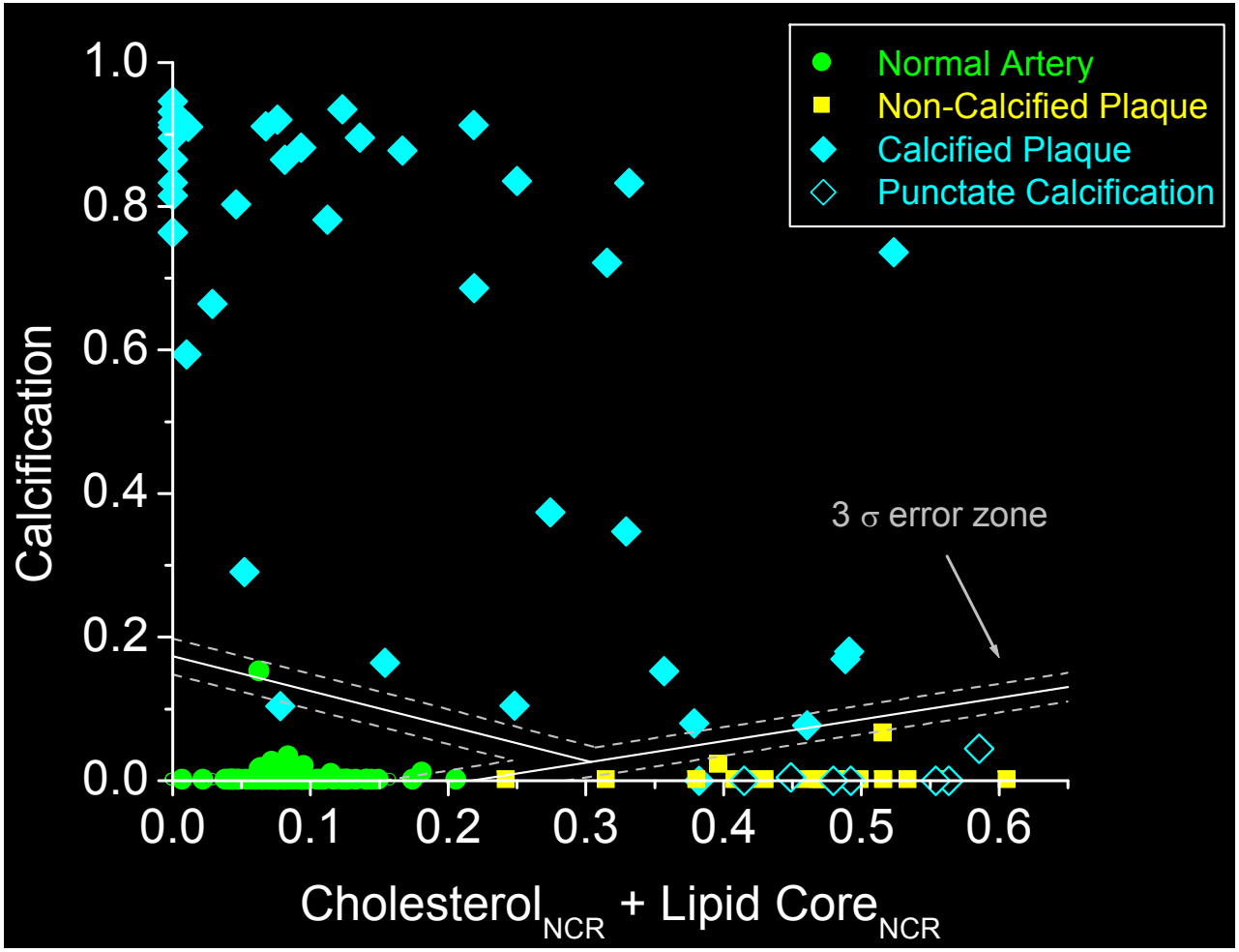


Image from medstat.med.utah.edu/WebPath/webpath.html

Cardiovascular Disease

- **Coronary Artery Disease Classification: A Prospective Study**



Buschman HPJ, Motz JT, et al. *Cardiovascular Pathology* **10**(2), 59-68 (2001)

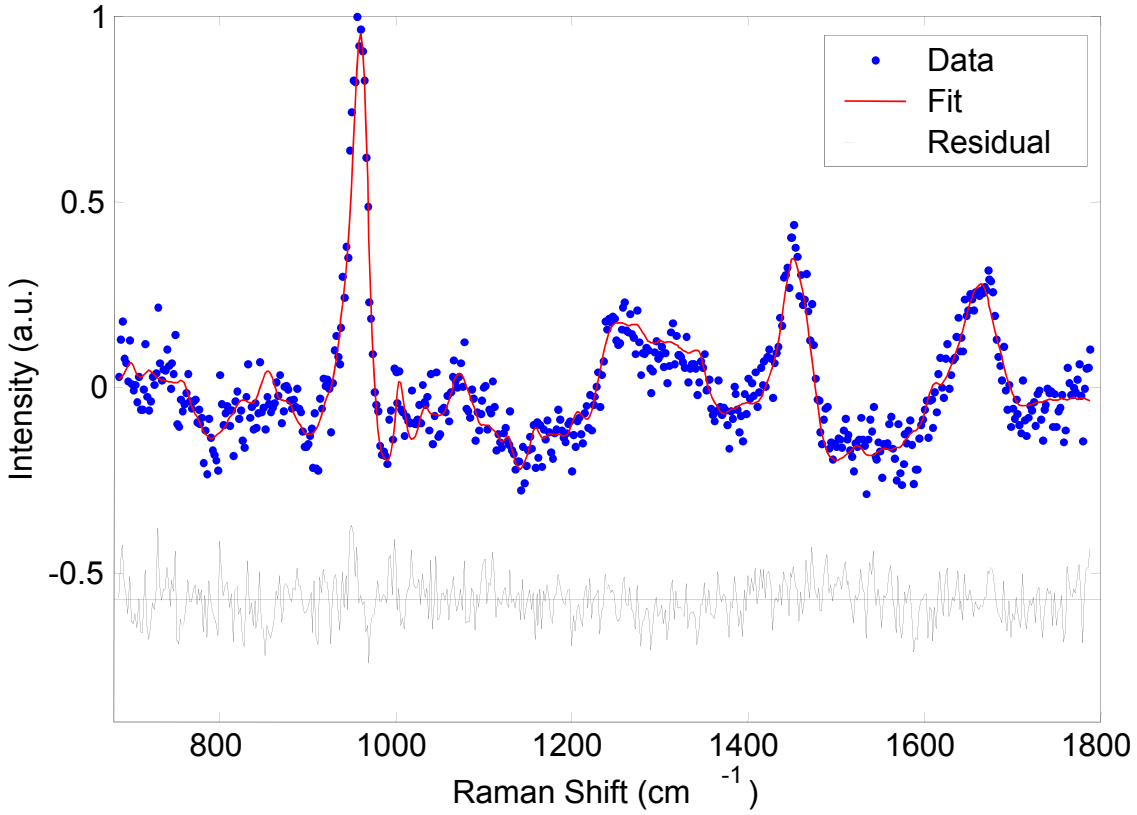
Cardiovascular Disease

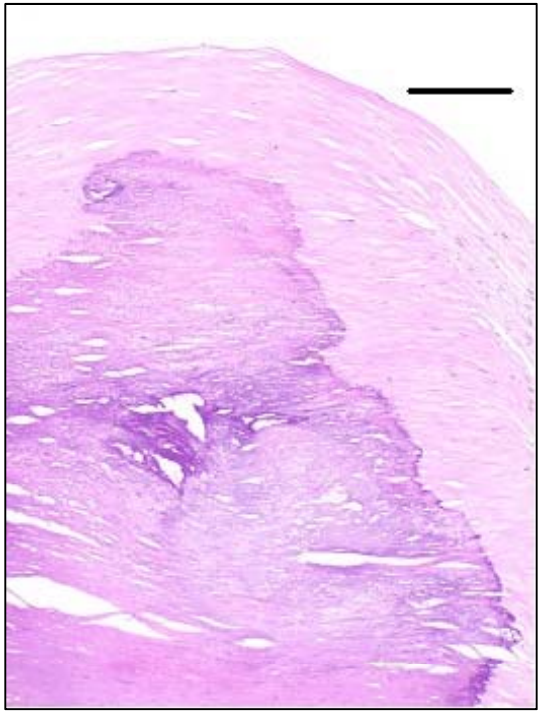
Clinical In Vivo Data: Methods

- **Peripheral vascular surgery**
  - Femoral bypass
  - Carotid endarterectomy
- **Laser power calibration set with Teflon**
  - ~100 mW (82-132mW)
- **OR room lights turned off as during angioscopy**
- **Spectra collected for a total of 5 seconds**
  - 20 accumulations of 0.25s each
  - Probe held normal to arterial wall
- **Analysis of 1s and 5s data**
  - Additional model components: sapphire, epoxy, water, HbO_2

Cardiovascular Disease

- **Clinical In Vivo Data: Calcified Plaque**

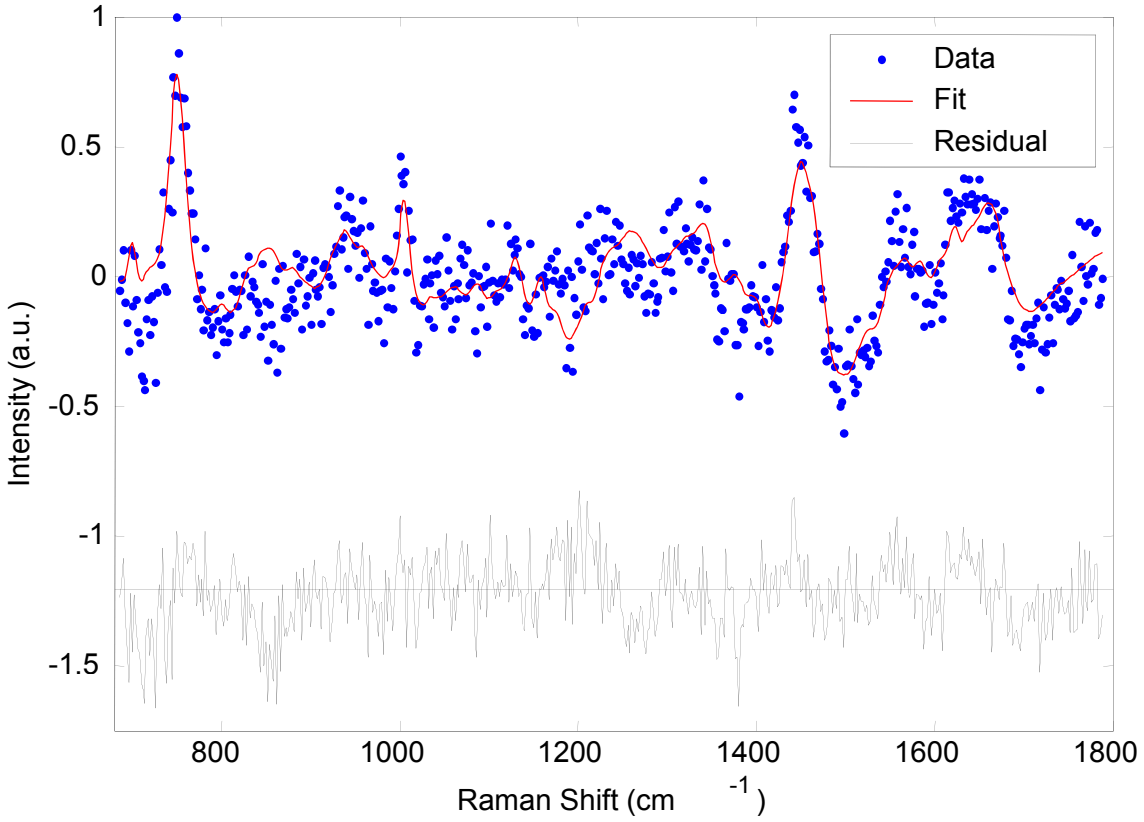


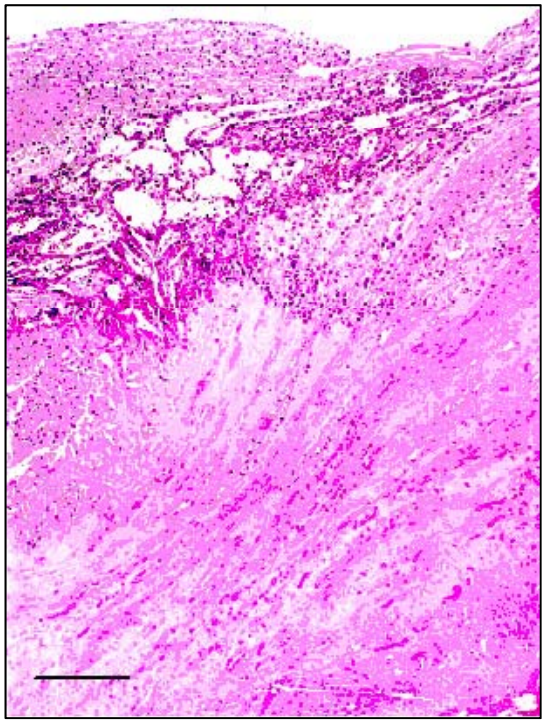


Motz JT *et al.*, J Biomed Opt **11**(2): 021003

Cardiovascular Disease

- **Clinical In Vivo Data: Ruptured Plaque**

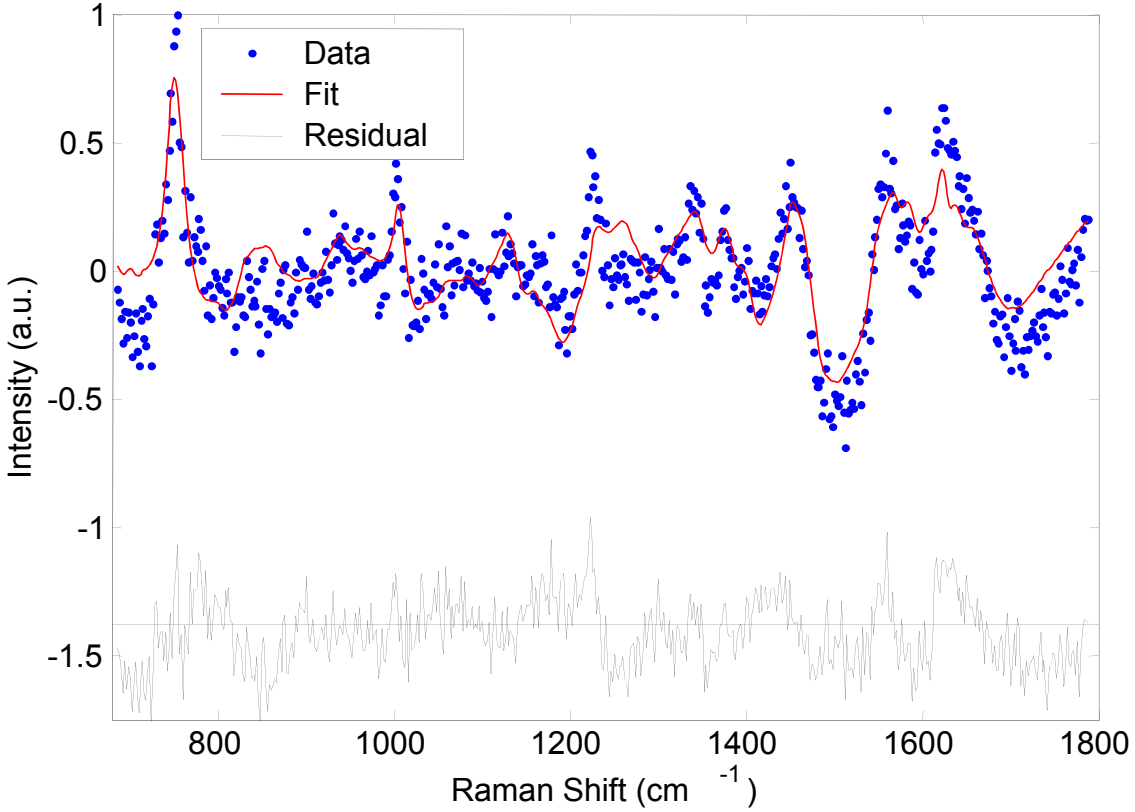


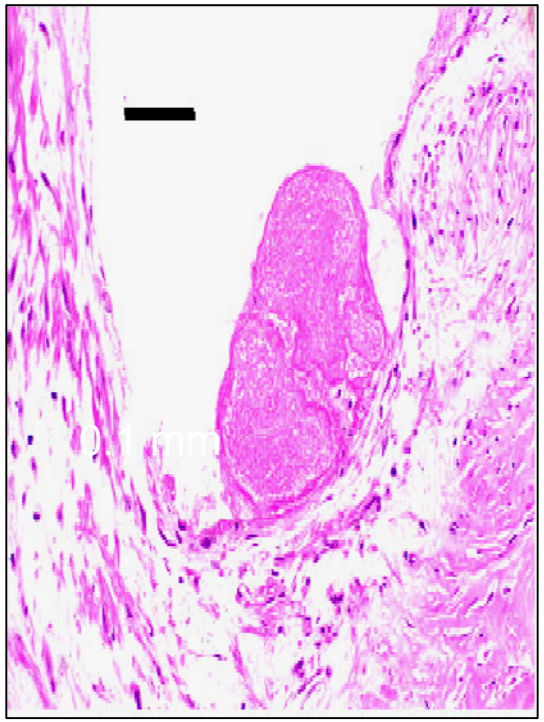


Motz JT *et al.*, J Biomed Opt **11**(2): 021003

Cardiovascular Disease

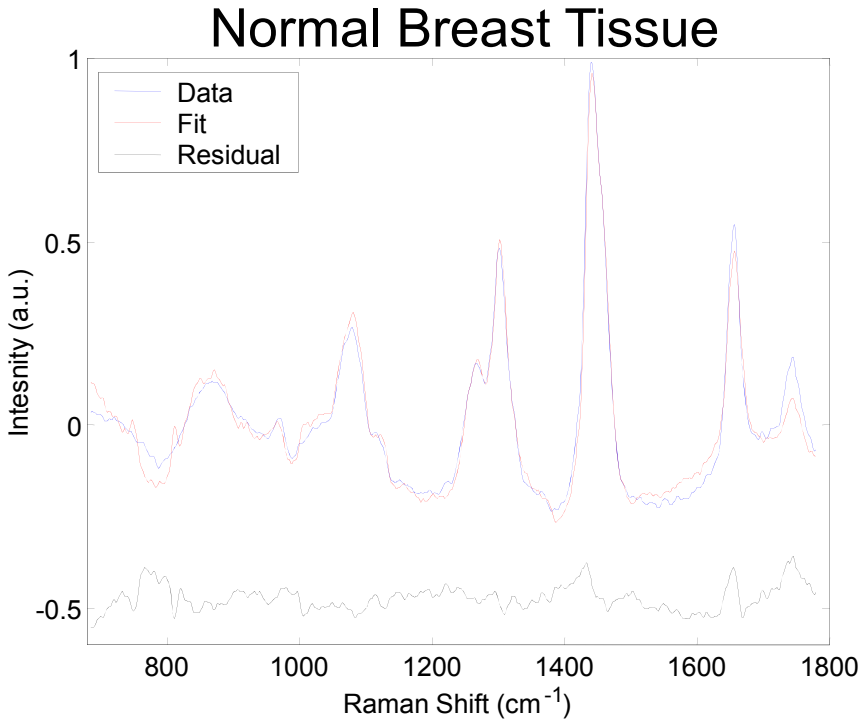
- **Clinical In Vivo Data: Thrombotic Plaque**

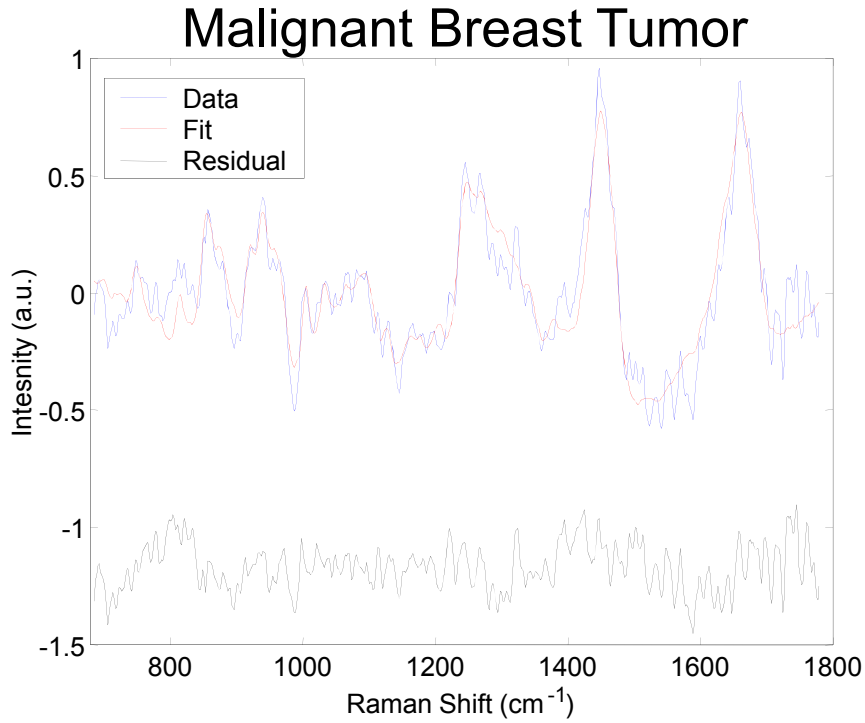




Motz JT *et al.*, J Biomed Opt **11**(2): 021003

Application To Other Diseases

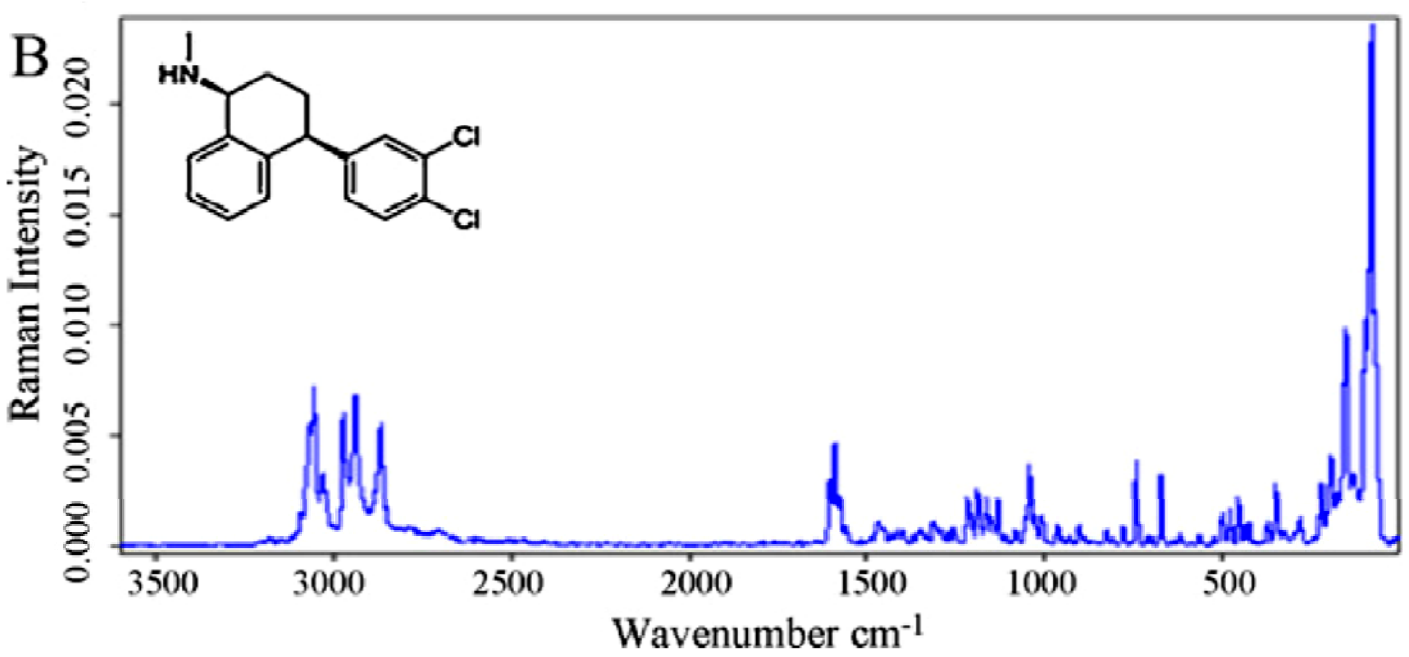




100 mW excitation, 1 second collection

Frontier Investigations

- **High-Wavenumber Raman**



Advantages

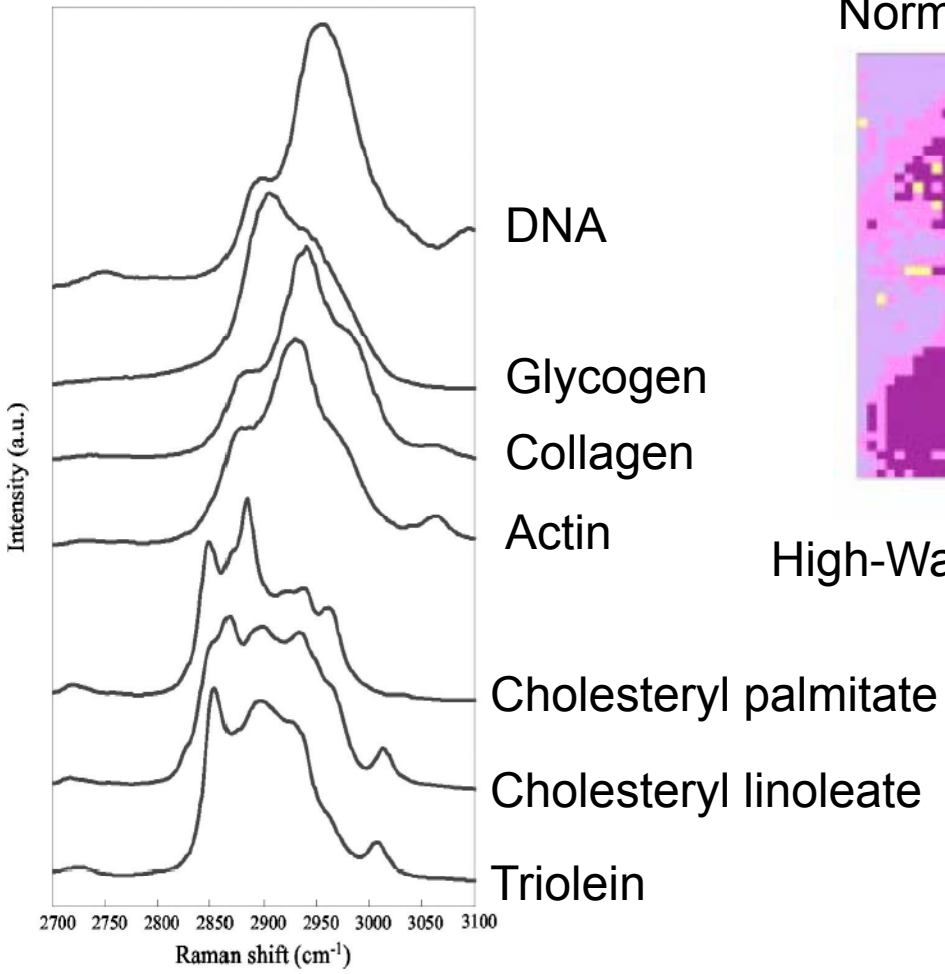
- Higher Raman signal
- Lower fluorescence
- No fiber background
- Distinguishes cholesterol esters

Disadvantages

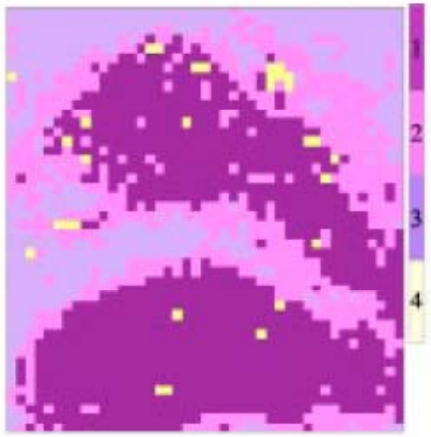
- Broader lineshapes
- Smaller spectral region
- Mostly limited to lipids
- No calcification signalc

Frontier Investigations

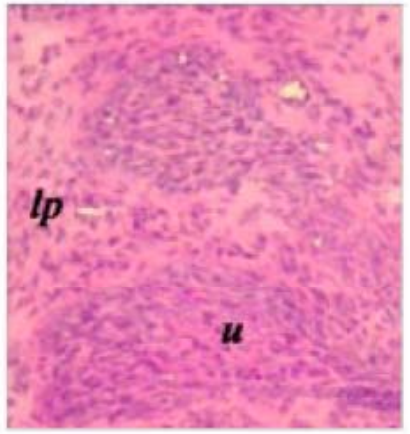
- **High-Wavenumber Raman**



Normal Bladder



High-Wavenumber



H&E

lp: lamina propria
u: urothelium

Koljenovic S *et al.*, J Biomed Opt **10**(3): 031116 (2005)

Conclusions

- Raman spectroscopy ‘fingerprints’ molecules by characterizing interactions between photons and molecular vibrations
- Near-infrared excitation is preferred for biomedical applications
- Recent optical fiber probe developments allow accurate real-time analysis in vivo
- New areas of research are promising for widespread clinical application

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References

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