



SERS, CARS, IR, FTIR

Variations of Raman Spectroscopy



- **Non-resonant Raman spectroscopy**
 - Visible (stronger signal)
 - Near-infrared (less fluorescence)
- **(UV) Resonance Raman spectroscopy**
- **Raman microscopy/imaging**
- **Fiber optic sampling**
- **Time resolved (pulsed) Raman spectroscopy**
- **High-wavenumber Raman spectroscopy**
- **Surface Enhanced Raman Spectroscopy (SERS)**
- **Non-linear Raman spectroscopy**
 - CARS: Coherent Anti-Stokes Raman Spectroscopy

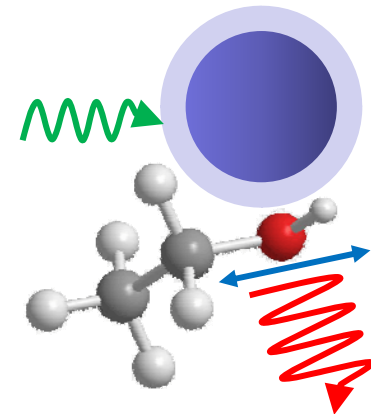
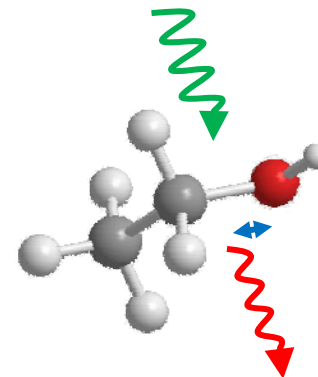
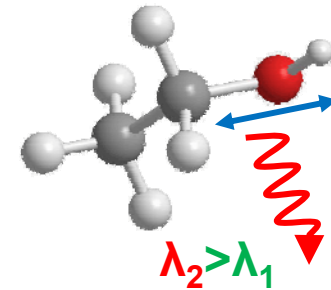
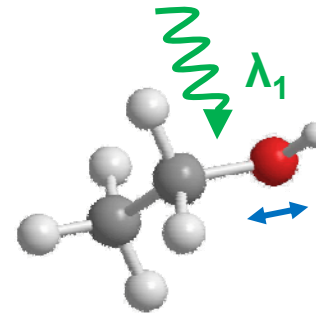


- **Raman Limitations**

- Raman intensity lines are 0.001% ($1:10^{-5}$, at most) of the source intensity.

- **Surface Enhanced Raman Spectroscopy (SERS)**

- Sample adsorbed on the surface of colloidal metal particles
- The intensity can be increased by $10^3 - 10^6 (10^{14})$
- Enhancement due to Surface Plasmons





• Surface Enhanced Raman Spectroscopy (SERS)

1928 C.V. Raman discovers “Raman Effect” of inelastic scattering



1974 Discovery of enhanced Raman signals (10^5 - 10^6) from molecules adsorbed on roughed Ag surfaces. Mechanism is attributed to enhanced surface area for adsorption.



1977 Debate begins over the exact mechanism of signal enhancement.



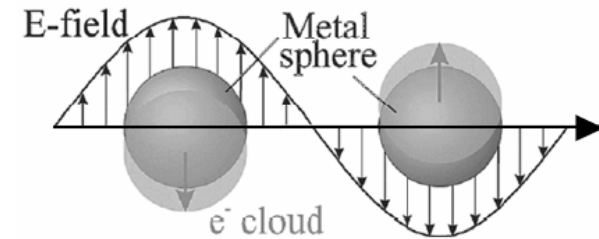
- M. Fleischmann, *et al.*, *Chem. Phys. Lett.*, **26** 163 (1974)
 D.L. Jeanmaire, R.P. Van Duyne, *J. Electroanal. Chem.*, **84** 1 (1977)
 M.G. Albrecht, J. A. Creighton, *J. Am. Chem. Soc.*, **99** 15 (1977)
 S. Schultz, *et al.*, *Surface Science*, **104** 419 (1981)
 M. Moskovits, , *Reviews of Modern Physics*, **57** 3 (1985)
 K. Kneipp, *et al.*, *Chem. Rev.*, **99** 2957 (1999)

SERS

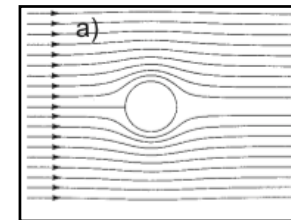


How does SERS work?

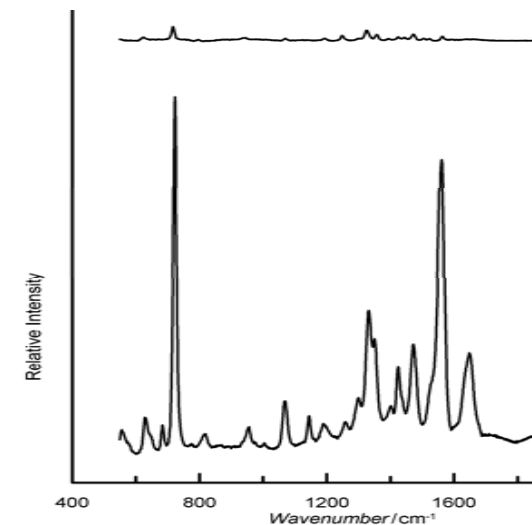
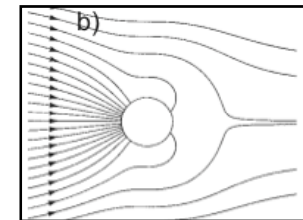
- **The mechanism of SERS is not completely understood.**
 - Electromagnetic enhancement
 - Chemical enhancement
- **Electromagnetic enhancement**
 - (Proposed by Jeanmarie and Van Duyne in 1977)
 - Arises from the presence of surface plasmons on the substrate.
 - Surface plasmons are electromagnetic waves that propagate along the surface parallel to the metal/dielectric interface.
 - Surface plasmons are generated when the incident light excites the electron gas of the metal.
 - When a substrate is placed in the proximity of the plasmon, it experiences an enhanced electromagnetic field and produces an enhanced scattered Raman field.
- **Chemical enhancement**
 - (Proposed by Albrecht and Creighton in 1977)
 - Involves charge transfer between the chemisorbed species and the metal surface
 - This enhancement is generally less than a factor of 10



Away from plasmon resonance



At plasmon resonance





- **Drude's Model for Dielectric Constant in Metals**

$$m_e \frac{\partial^2 \vec{r}}{\partial t^2} + m_e \Gamma \frac{\partial \vec{r}}{\partial t} = -e \vec{E}$$

Where m_e is electron mass, e is its charge, Γ a damping constant

- Also

$$E = E_0 e^{-j\omega t} \quad r = r_0 e^{-j\omega t}$$

- Then

$$\omega_p = \sqrt{\frac{Ne^2}{m_e \epsilon_0}} \quad \epsilon(\omega) = \frac{\epsilon_{\text{target}}(\omega)}{\epsilon_{\text{medium}}(\omega)} = 1 - \frac{\omega_p^2}{\omega[\omega + i\Gamma]} \quad \text{Re}\{\epsilon(\omega)\} = 1 - \frac{\omega_p^2}{[\omega^2 + \Gamma^2]}$$

Where N =number density of electrons, ϵ_0 =the permittivity of free space or electric constant ($8.85419 \times 10^{-12} \text{ Fm}^{-1}$)

- The induced field (for a metal sphere) is

$$E_{\text{induced}} = \left\{ \frac{[\epsilon_m(\omega) - \epsilon_h]}{[\epsilon_m(\omega) + 2\epsilon_h]} \right\} E_{\text{laser}}$$

where $\epsilon_m(\omega)$ is the complex dielectric function of the metal and ϵ_h is the relative permittivity of the surroundings

- Resonance $\rightarrow \boxed{\text{Re}\{\epsilon_m\} = -2\epsilon_h}$ and $\boxed{\text{Im}\{\epsilon_m\} \rightarrow 0}$

- The factor “2” changes for different shapes

Complex Index of refraction $m = n - jk$

Complex dielectric constant $\epsilon = \epsilon_1 + j\epsilon_2$

$$n = \sqrt{\frac{(\epsilon_1^2 + \epsilon_2^2)^{1/2} + \epsilon_1}{2}} \quad k = \sqrt{\frac{(\epsilon_1^2 + \epsilon_2^2)^{1/2} - \epsilon_1}{2}}$$

$$\epsilon_1 = n^2 - k^2 \quad \epsilon_2 = 2nk$$

SERS



- **SERS substrates commonly used**

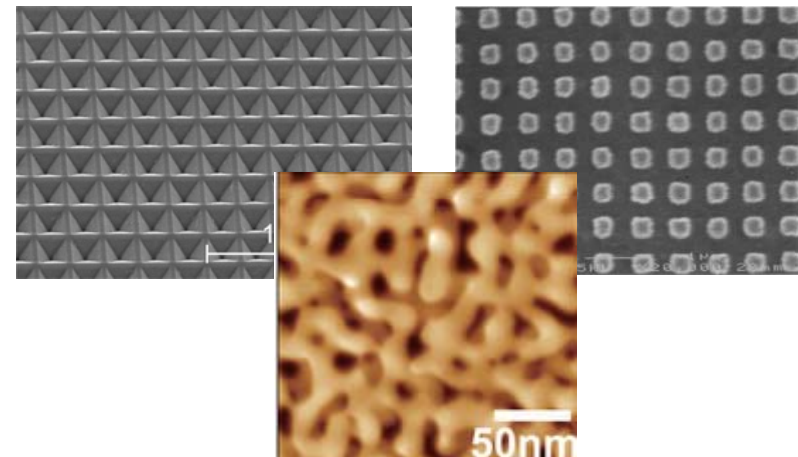
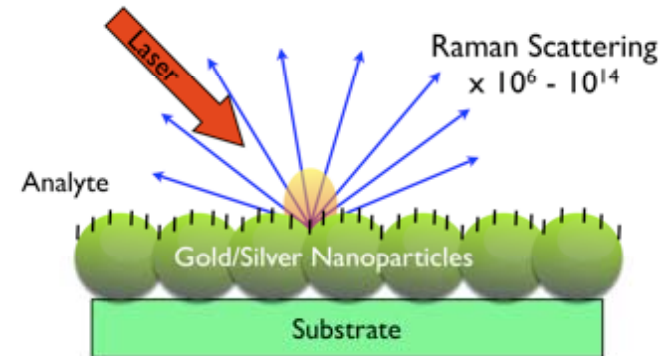
- Silver (Ag), gold (Au) and copper (Cu)
- The energy required to generate plasmons matches the light sources typically used in Raman spectroscopy

- **Metal nanoparticles**

- Spheres
- Rods
- Nanoshells

- **Surface preparations**

- Chemically etched
- Nano lithography,
- Electron beam lithography
- Imprint
- Largest enhancements for rough surfaces of 10 – 100 nm

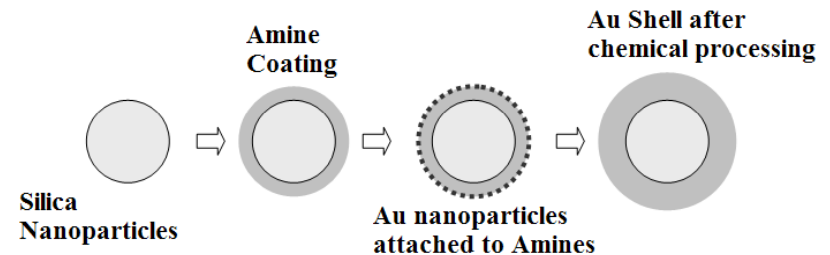


SERS

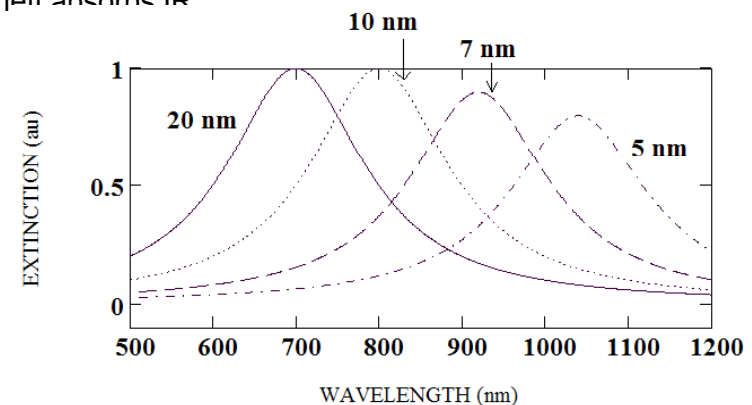


• Nanoshells

- Dielectric sphere coated with a nanoshell of metal
- Core materials → AuS or silica
 - Radius (r) between 30-250 nm
- Shell → metal (e.g. gold)
 - Thickness (t) → 10-30 nm.
- Plasmon resonance a function of the two dimensions (r & t)
- Fabrication
 - Dielectric sphere coated with a layer of amines
 - Binds 1-2 nm gold colloids from suspension.
 - Chemical treatment with HAuCl₄ in the presence of formaldehyde
 - Results in an additional layer of gold



Vial on the left has solid gold colloids. Others have colloids with metal nano-shells with decreasing thickness. Vial on left absorbs IR

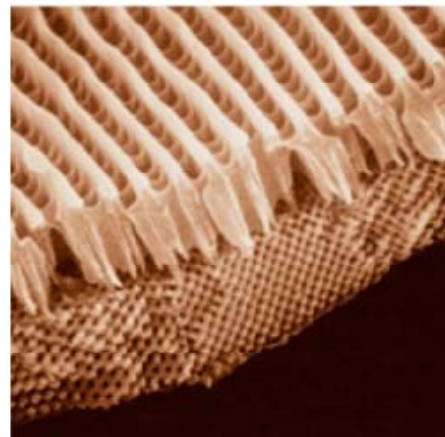


Plasmon resonance extinction for core-shell nanospheres of various shell thickness. Core radius is 60 nm



• Nanostructures in nature and art

Photonic Nanocrystals → colors in butterfly wings (a regular array of scattering elements. They reflect the same wavelength of light irrespective of incident angle



Yablonovitch, Sci.Am. 2001

Plasmon absorption by metallic nanoparticles results in color of stained glass windows, glass cups, ceramic pots





- **Why use SERS?**

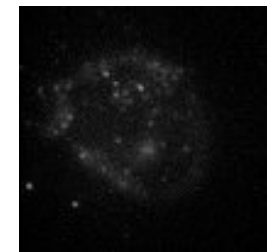
- High sensitivity and Specificity
- Low-power lasers and low magnification optics are suitable to acquire SERS spectra in very short acquisition times (typical ~ 10 s).

- **Many applications**

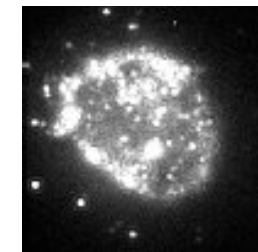
- Molecular fingerprinting
 - Unique vibrational spectra distinguishes molecules
- Tagless biosensing
 - Fluorescent dyes are not needed
- Multiplexed sensing
 - Plasmon resonances allow for sensor tunability
- In vivo applicability
 - Near-IR excitation and biocompatibility
- Femtomolar and beyond
 - Single molecule spectroscopy is possible
- Nanoprobes can be multimodal



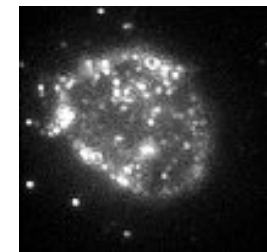
1500 cm^{-1}



1532 cm^{-1}



1600 cm^{-1}



1635 cm^{-1}



- **Cancer imaging with SERS**

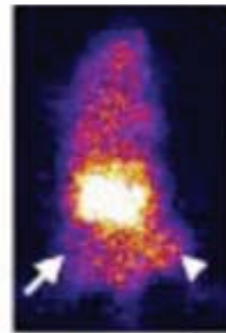
- Nanoparticles attached to a tumour-seeking antibody or peptide
- Tracked as they move around the body

Imaging with Dual-Labeled Probes

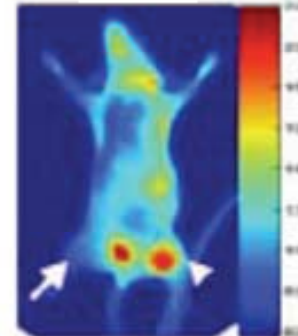
White-Light



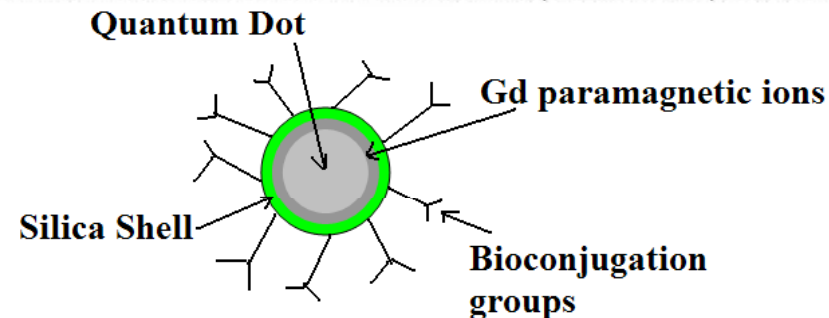
γ - Scintigraphy



NIR



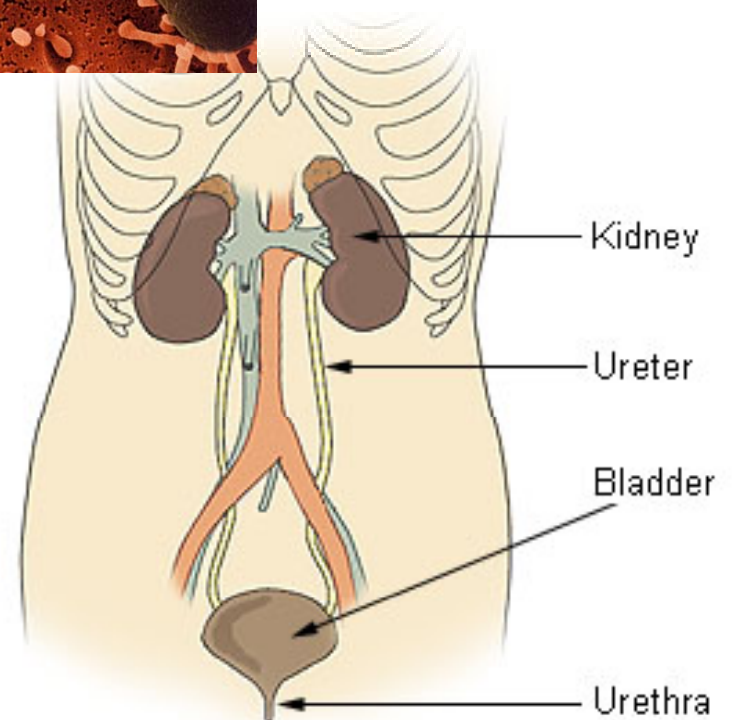
Multifunctional Nanoprobes-Louie: EAD289-Topics in Biophotonics, UC Davis





- **Diagnosis and Antibrogram for Urinary Tract Infection (UTI)**

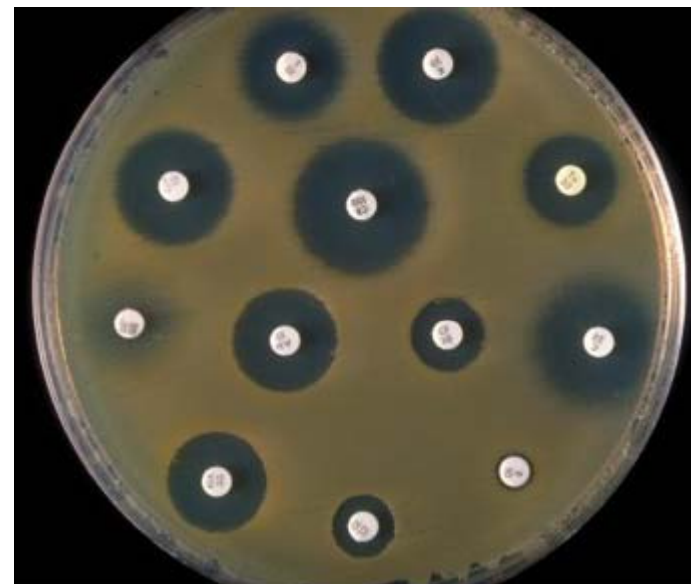
- UTI : An infection anywhere in the urinary tract
- Caused by bacteria in the digestive tract, vagina, or around the urethra (Commonly due to *E. coli*)
- Affects mostly: women, chronically ill patients
- One of the most common types of infections
 - 34% of adults report as having had at least one UTI
 - 1 in 2 women and 1 in 7 men will develop a UTI in their lifetime





- **Diagnosis and Antibigram for Urinary Tract Infection (UTI)**

- Identification of bacteria with cultures: 24h
- Specific antibiotic can be determined after antibiogram: another 24 h





- **Complete urinary tract infection (UTI) diagnosis and antibiogram using SERS**

1. Identification of positive and negative samples for UTI
2. Classification of causative bacteria
3. Determination of antibiotic sensitivity

- **Advantages**

- Minimal sample preparation
- Inexpensive
- Rapid

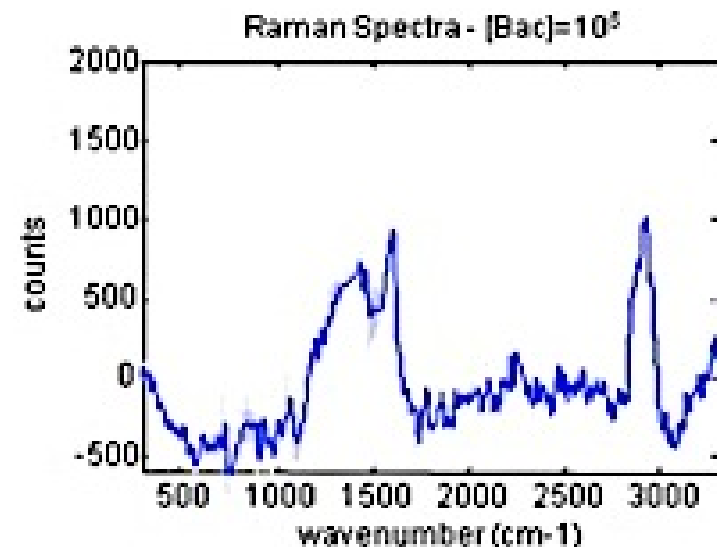
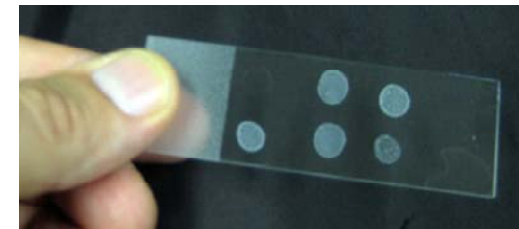


SERS



- **Complete urinary tract infection (UTI) diagnosis and antibiogram using SERS**

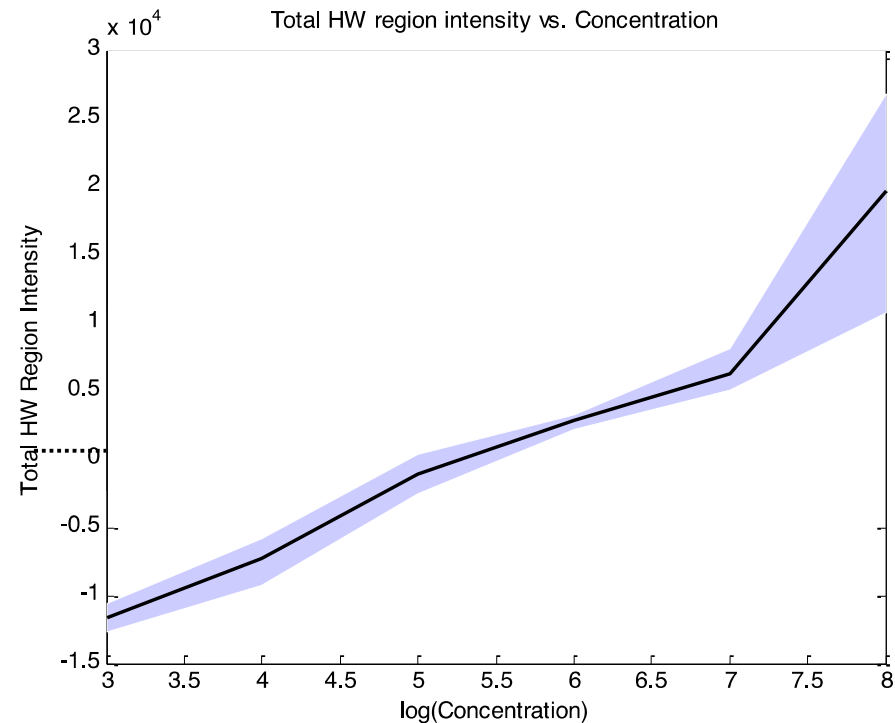
- Bacterial isolates from patients with UTI (n=50)
 - E. coli, Klebsiella p., Proteus spp., Enterococcus spp., Citrobacter spp.
- SERS:
 - 785 nm laser
 - 180 mW power
 - 4.5 cm⁻¹ resolution
 - Data acquisition: 20s x 30
 - Raman spectra collected with gold NP





1. Identification of sample as positive or negative for UTI using SERS

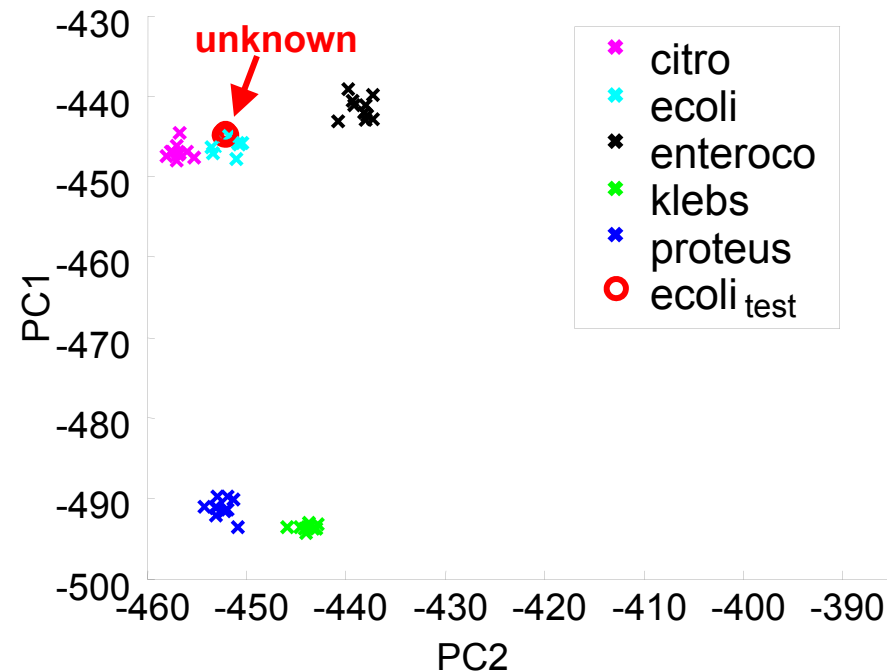
- Total high-wave region intensity correlates with log concentration
 - After band-pass filtering some values may be negative
- Samples with $I > -500$ are considered positive





2. Classification of Causative Bacteria Using SERS

- Analysis
 - Feature vector: Ratios of spectral windows
- Classification
 - Principal components transformation
 - Linear Discriminant Analysis
 - Leave-one-out cross validation

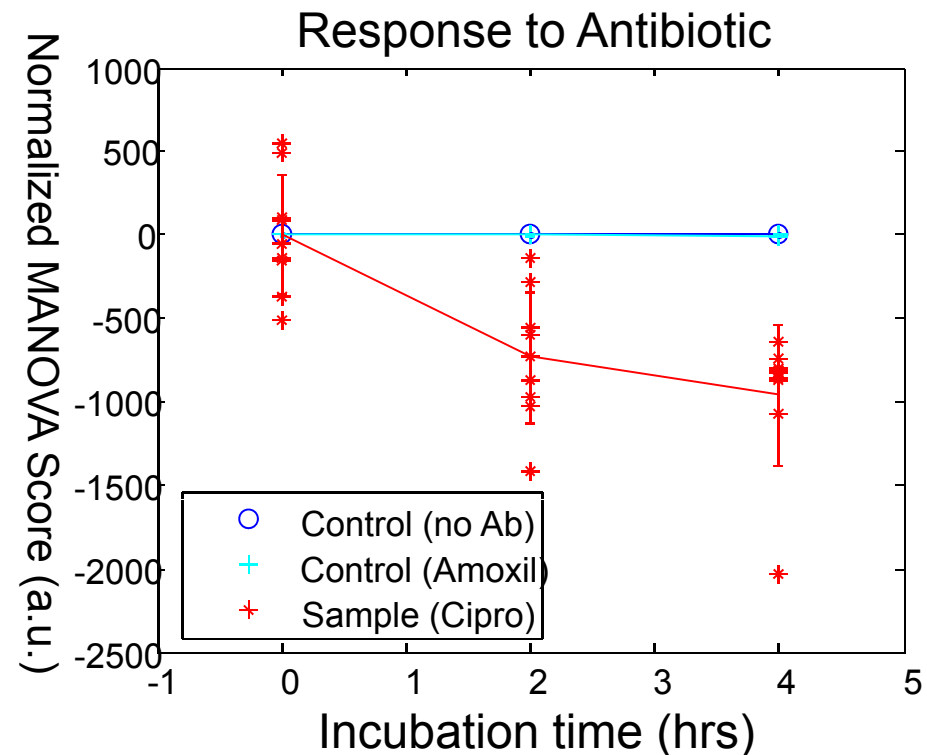


Classes	Correct Classification
3	28/30 (93%)
5	42/46 (91%)



3. Antibiotic Sensitivity Testing Using SERS

- Analysis
 - Principal component transformation
 - MANOVA
 - T-test
- Distributions differentiate after 2-4 hours.
- Modifications in the structure of the bacteria?



Surface Plasmon Resonance

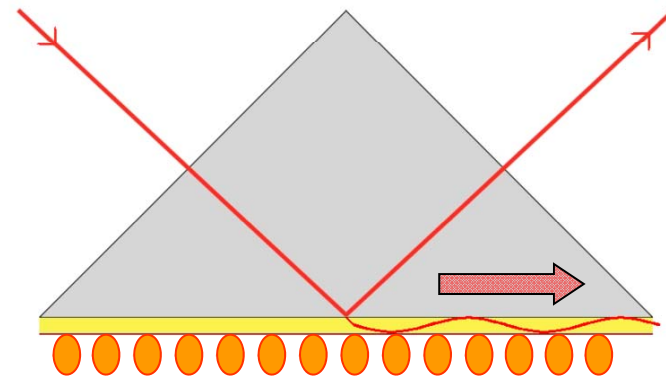
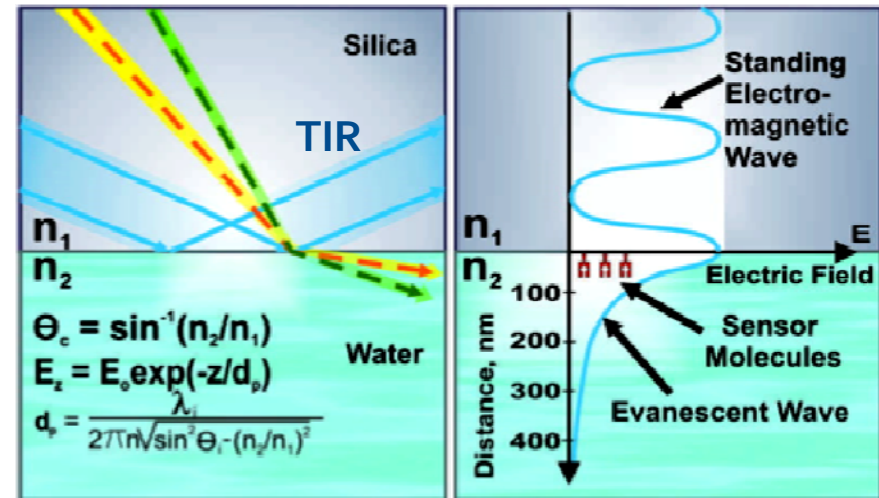


- **Surface plasmon waves**

- Can be generated optically on bulk surface at the interface of metal and dielectric
- Special excitation geometry is required to produce such a wave
- The traveling wave is associated with a wavevector $k(sp)$.

$$k(sp) = kn \sin(\theta)$$

- Where, $k(sp)$ is the wavevector of the surface plasma wave and $k=(2\pi/\lambda)$ that of the light wave

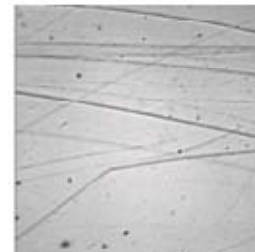
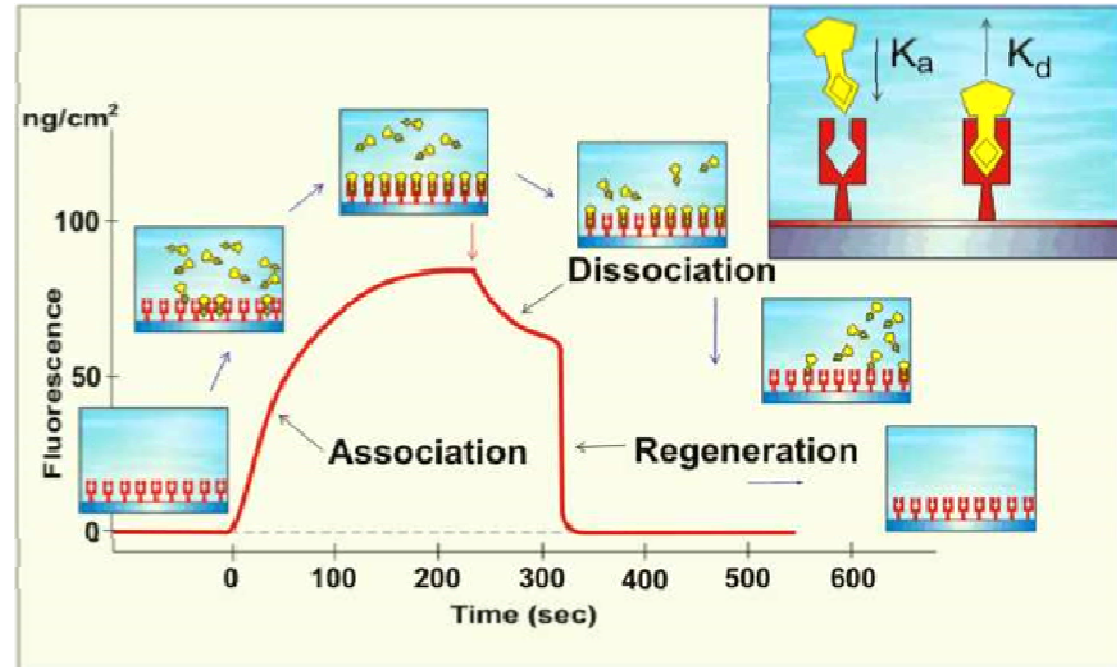


Kretschmann configuration

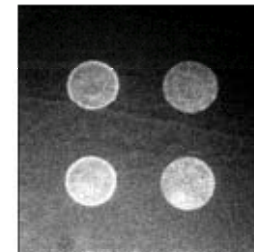
Surface Plasmon Resonance



- **Typical TIRF Sensogram**
- **Advantages**
 - High Signal to noise ratio (very little secondary emission from bulk solution)
 - Highly robust, low cost, portable
- **Drawbacks**
 - Need for labels
 - High cross-reactivity (hence not easy to multiplex)



Epifluorescence



TIRF

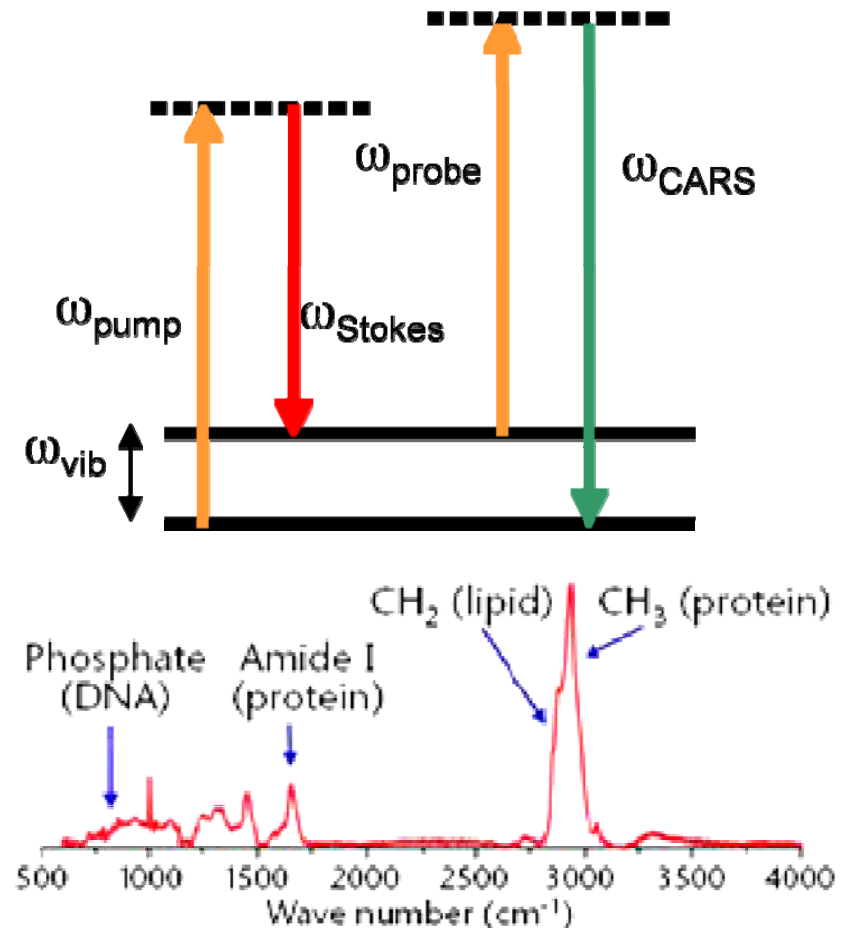
CARS



- **Coherent Anti-Stokes Raman Spectroscopy (CARS)**

- Non linear Raman process
- Generated at the focus of the beam
- $\omega_{AS} = 2\omega_p - \omega_s$
- Signal is coherent

$$P_{as} = \chi^{(3)} E_p^2 E_s^* \quad I \propto (I_p)^2 I_s$$

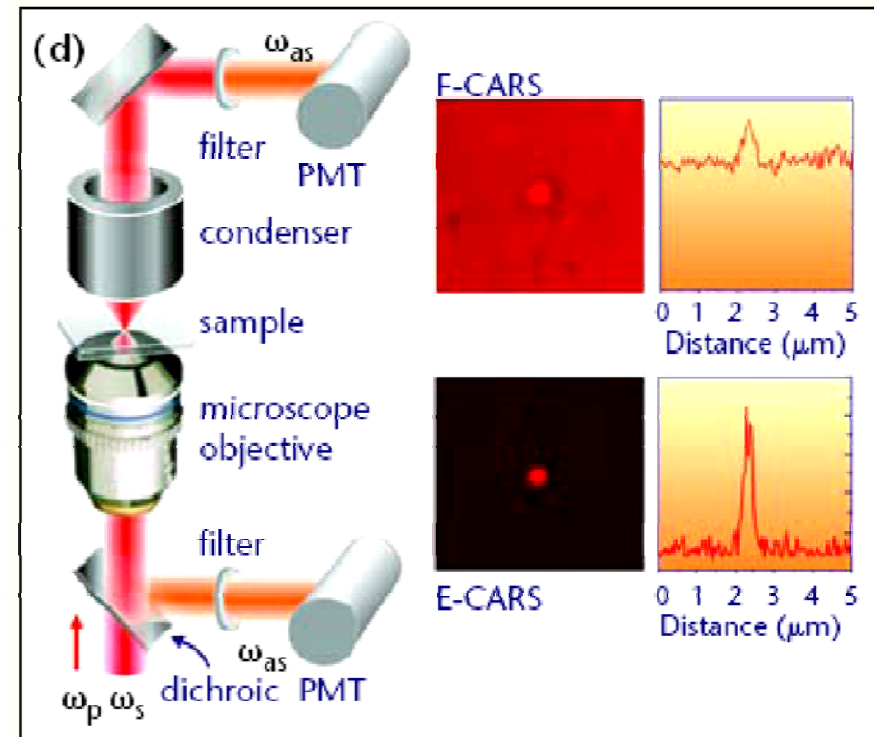


CARS



• CARS Microscopy

- Two lasers at different frequencies
- ps or fs pulse trains with high peak Intensity
- Two beams spatially overlapped at focii
- Two beams temporally overlapped





- **Advantages**

- Intrinsic vibrational contrast
- Strong, directional signal → Sensitive
- Requires moderate average powers good for biological samples
- Only generated at focus → 3D sectioning capability
- Higher in frequency than one-photon fluorescence → easily detected in presence of a strong fluorescent background.
- Near IR
 - Little scattering → deep penetration in tissues
 - Little absorption → Low photodamage

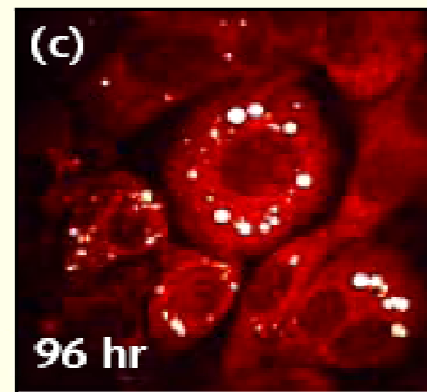
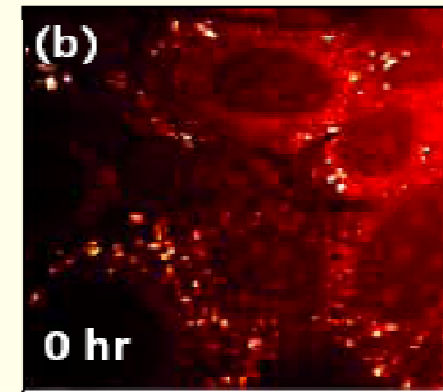
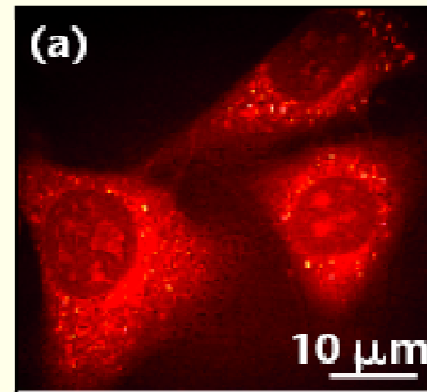
- **Disadvantages**

- Non resonant background from bulk very strong
- Expensive laser sources
- Have to know beforehand the vibrational band of interest
- Currently limited tunability of sources – improving in line with the laser sources....



• Applications

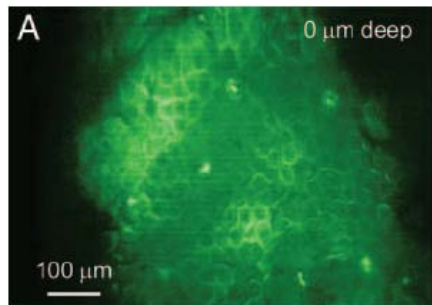
- Sensitive probe for lipids
- Lipid bilayer, thin objects, small objects
- Fast dynamic scanning of processes in living cells
- In vivo capabilities



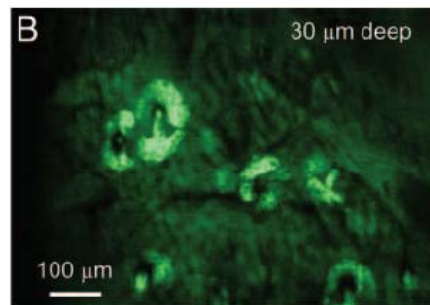


Images of a hairless mouse ear

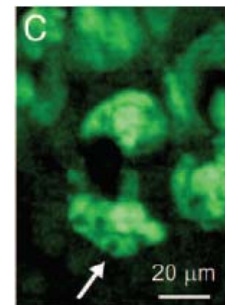
Chemical imaging of tissue in vivo with video-rate coherent anti-Stokes Raman scattering microscopy C. Evans et al. *Proc. Natl. Acad. Sci.* 102 (46) 16807 (2005)



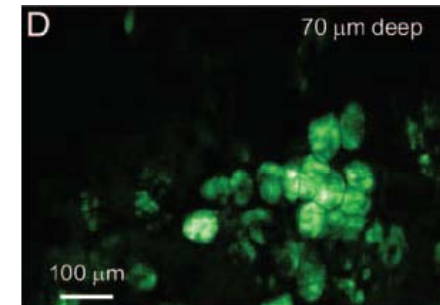
Stratum corneum with bright signals from the lamellar lipid intercellular space that surrounds the polygonal corneocytes. Bright punctuated dots are ducts of sebaceous glands.



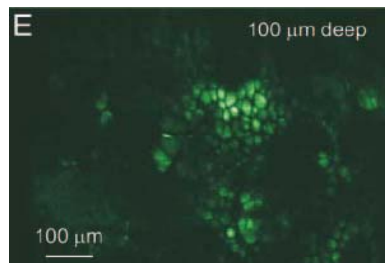
Sebaceous glands at ~30 μm from skin surface.



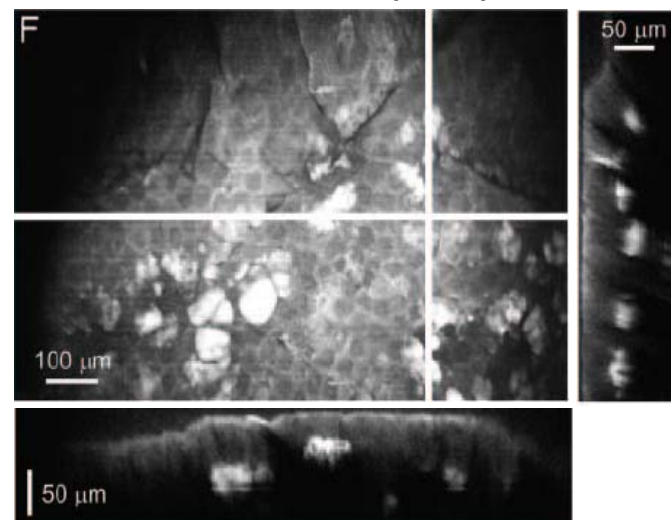
Individual cells of the gland compartment can be recognized, with nuclei visible as dark holes (arrow).



Adipocytes of the dermis at ~60 μm from skin surface.

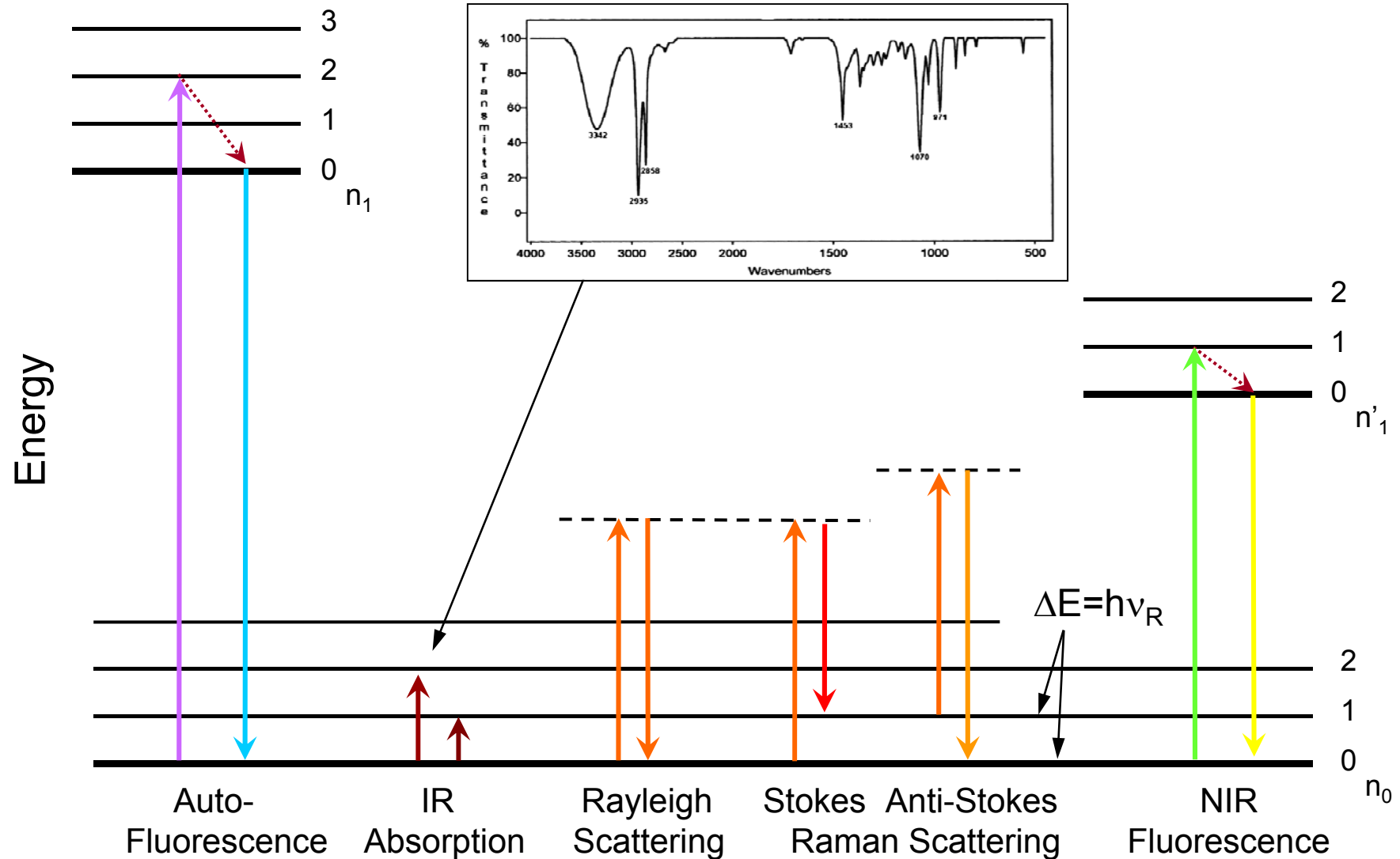


Adipocytes of the subcutaneous layer at a depth of ~100 μm.



2D projection of 60 depth-resolved slices separated by 2 μm. Panels to the right and under *F* show the *yz* and *xz* cross sections taken at the white lines, respectively.

Photo-Molecular Interactions

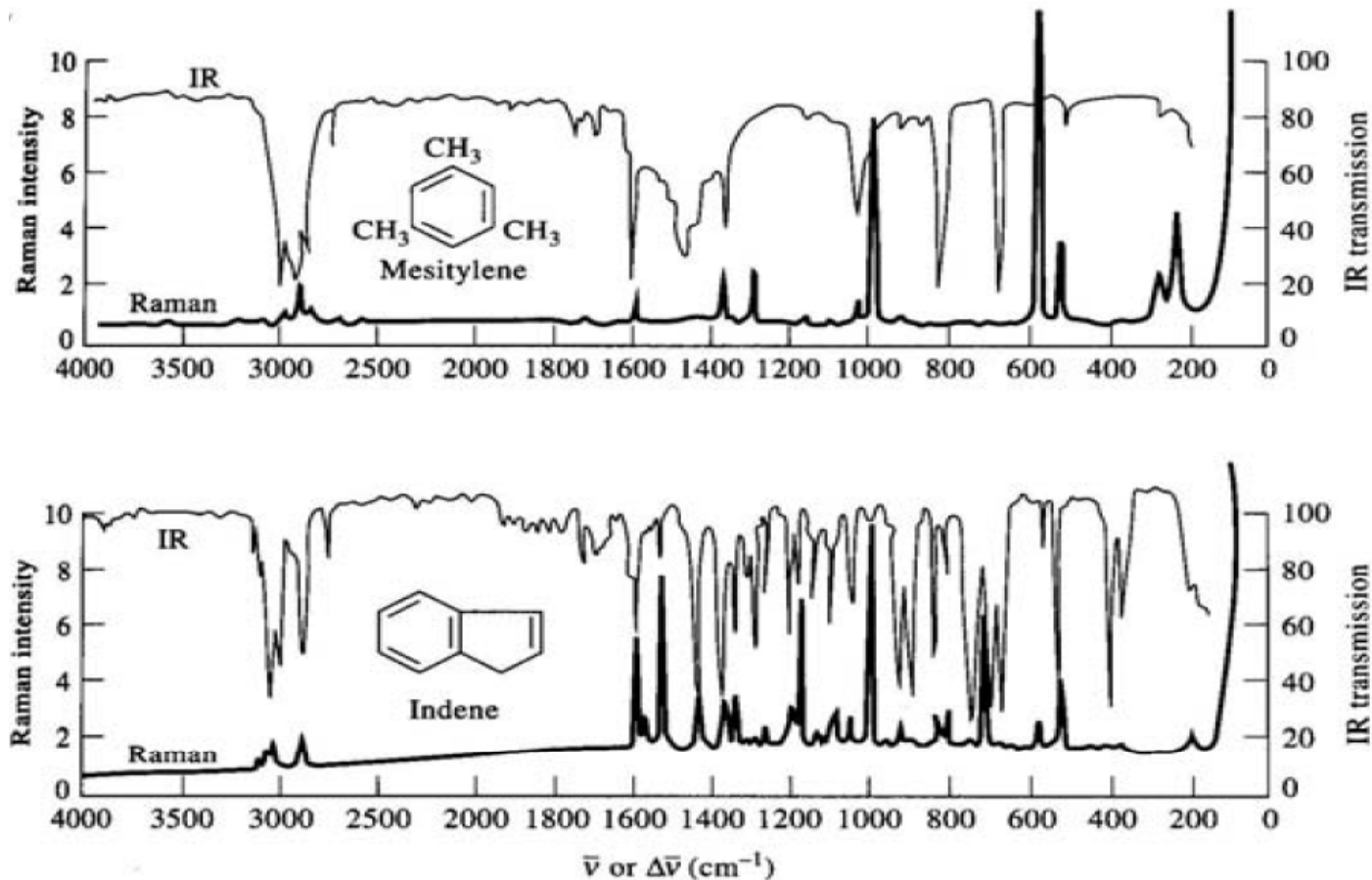


Infrared Absorption



- IR

- An absorptive process (vs. Raman which is a scattering process)
- Directly interrogates molecular vibrations



Infrared Absorption



- **IR and Raman are complementary techniques**

- Symmetric molecules with a center of inversion have vibrations which are either Raman or IR active, but not both (e.g. benzene)
- Molecules with no symmetry are active in both methods

(N)IR absorption	Raman
Absorption	Scattering
Requires change in dipole moment (No symmetric stretches observed, No diatomic activity)	Requires a change in polarizability with vibrational motion
Only observed in NIR and IR spectral regions	Occurs at all wavelengths
Strong signal	Weak signal
High water absorption	Water not a problem
Broad spectral features	Sharp spectral features for molecular fingerprinting
Requires some sample preparation in most cases	Does not require sample preparation

Infrared Absorption



	Near-IR	Mid-IR	Raman
Spectral range (cm ⁻¹)	13,300–3300	4000–400	4000–50
Analysis of:			
Gases	No	Yes	Yes
Liquids	Yes	Yes	Yes
Solids	Yes	Yes	Yes
Aqueous systems	Difficult	Very difficult	Yes
Macroscopic samples	Yes	Yes	Yes
Microscopic samples	No	Yes	Yes
Signal	Strong	Strong	Weak
Sampling	Easy	Difficult	Easy
Through glass windows	Yes	No	Yes
In situ	No	No	Yes
Quantitative	Yes	Difficult	Yes
Noninvasive	Yes	No	Yes
Fiber optic interfacing	Yes	No	Yes
Information content	Low. Limited to O–H, N–H, and C–H vibrations	High	High
Reaction monitoring and modeling	Requires chemometrics	Yes	Yes



- **Fourier Transform Infrared (FTIR) Absorption**

- Interferometric (instead of spectrograph-based) measurement
- Interferometer
 - An optical Fourier Transform on the emitted light
 - Modulates light emitted → an interferogram which has all IR frequencies encoded into it
- Advantages
 - The whole infrared spectrum is measured at high speed
 - Spectral range is continuously calibrated with HeNe laser
 - Fast, extremely accurate measurements
- Michelson Interferometer → OCT lecture

