

Técnicas de diagnóstico – espectroscopia Raman

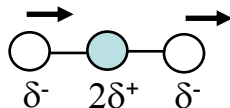
1.) Espectroscopia Raman: *complementar à espectroscopia IV (FTIR)*.

- radiação a uma certa frequência é espalhada pela molécula com deslocamentos no comprimento de onda referente ao feixe incidente.
- deslocamentos da frequência são relacionados à mudanças na vibração da molécula → associados com a absorbância no IV.
- Espectro Espalhamento Raman ~ Espectro Absorção IV
- Mecanismos diferem!

IV

i. modos vibracionais

ii. mudança no momento dipolar



iii. excitação da molécula para estado vibracional excitado

iv. vibrações assimétricas (ativas)

Raman

modos vibracionais

mudança em polarizabilidade decorrente de um campo externo aplicado



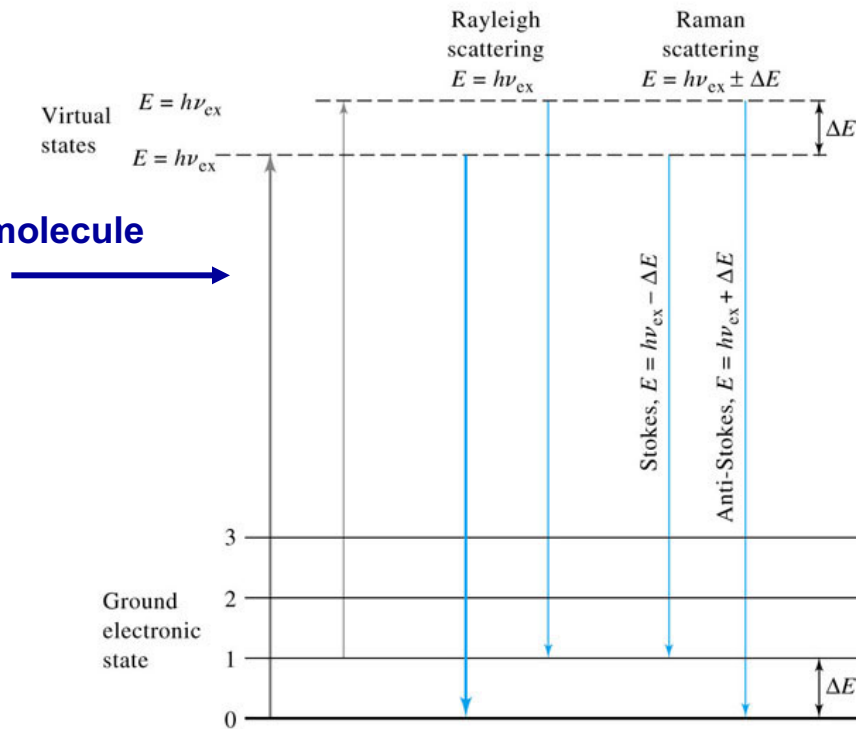
distorção momentânea dos elétrons distribuídos ao redor da ligação

vibrações simétricas (ativas)

2.) Princípios básicos:

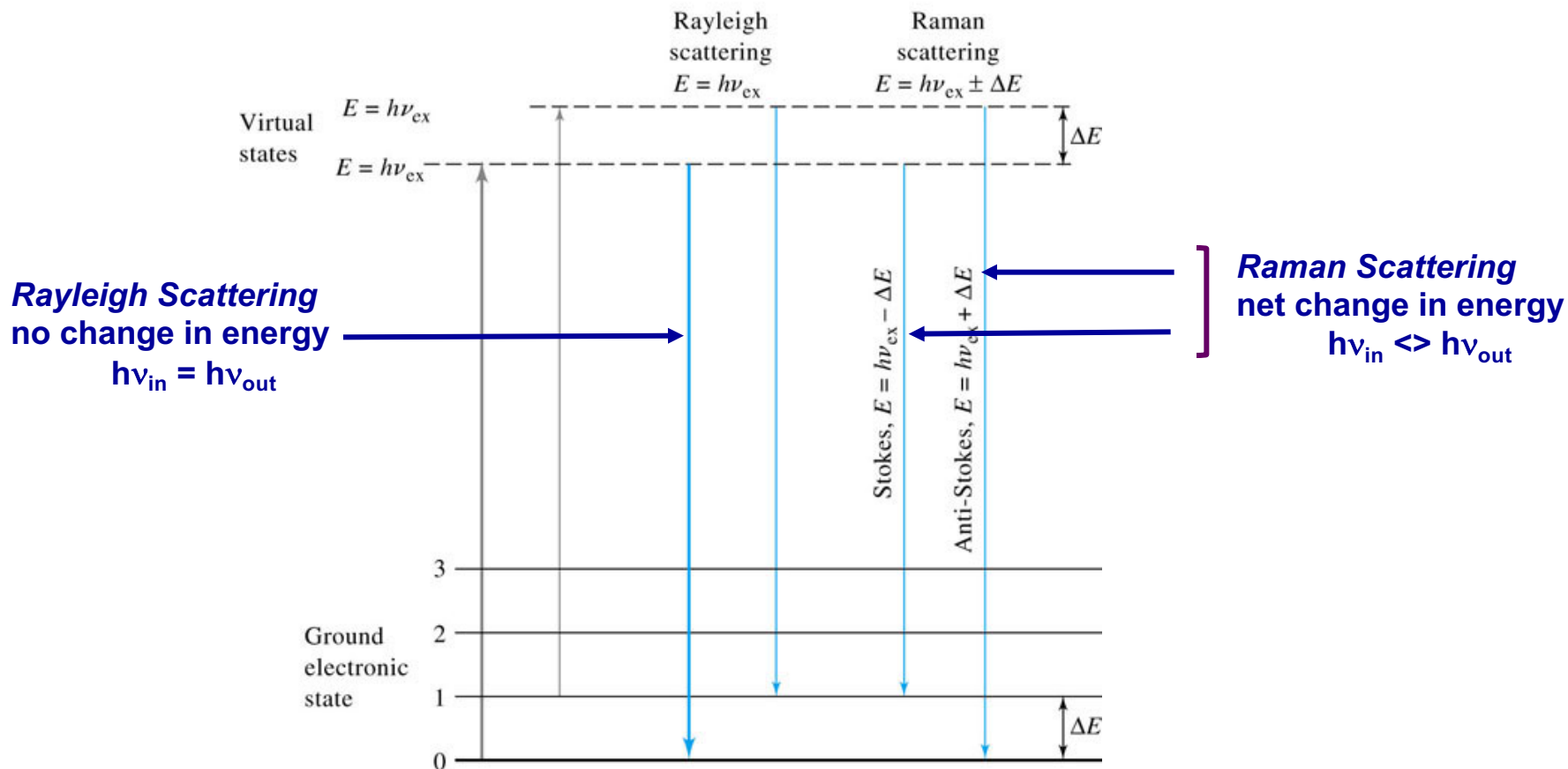
- luz é espalhada pela amostra em vários ângulos por uma absorção momentânea em um estado virtual e re-emissão

energy absorbed by molecule
from photon of light
not quantized



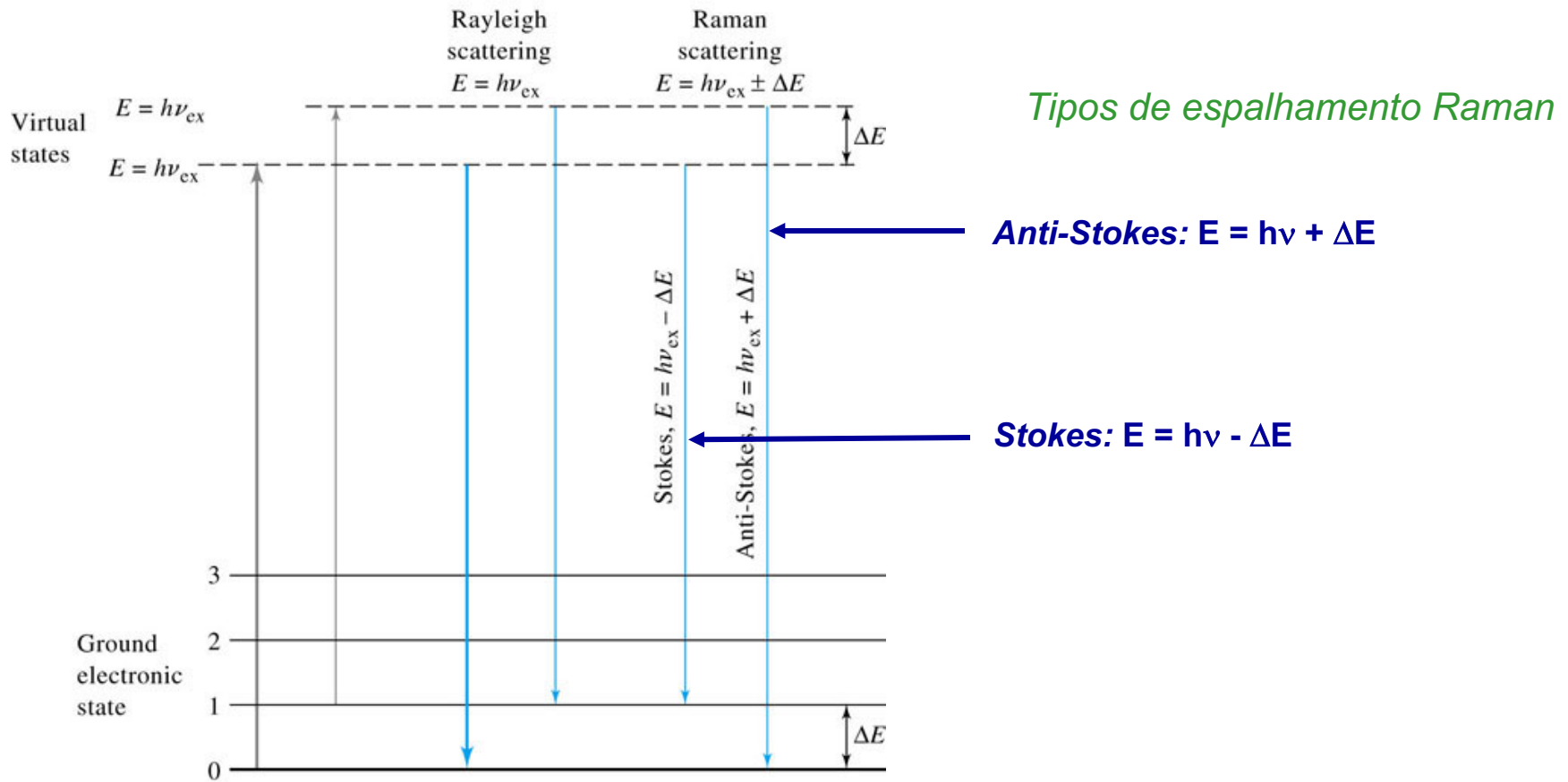
Infinite number
of virtual states

- processos de espalhamento com emissão na mesma energia do feixe incidente, outros com re-emissão com outras energias (diferente estado)



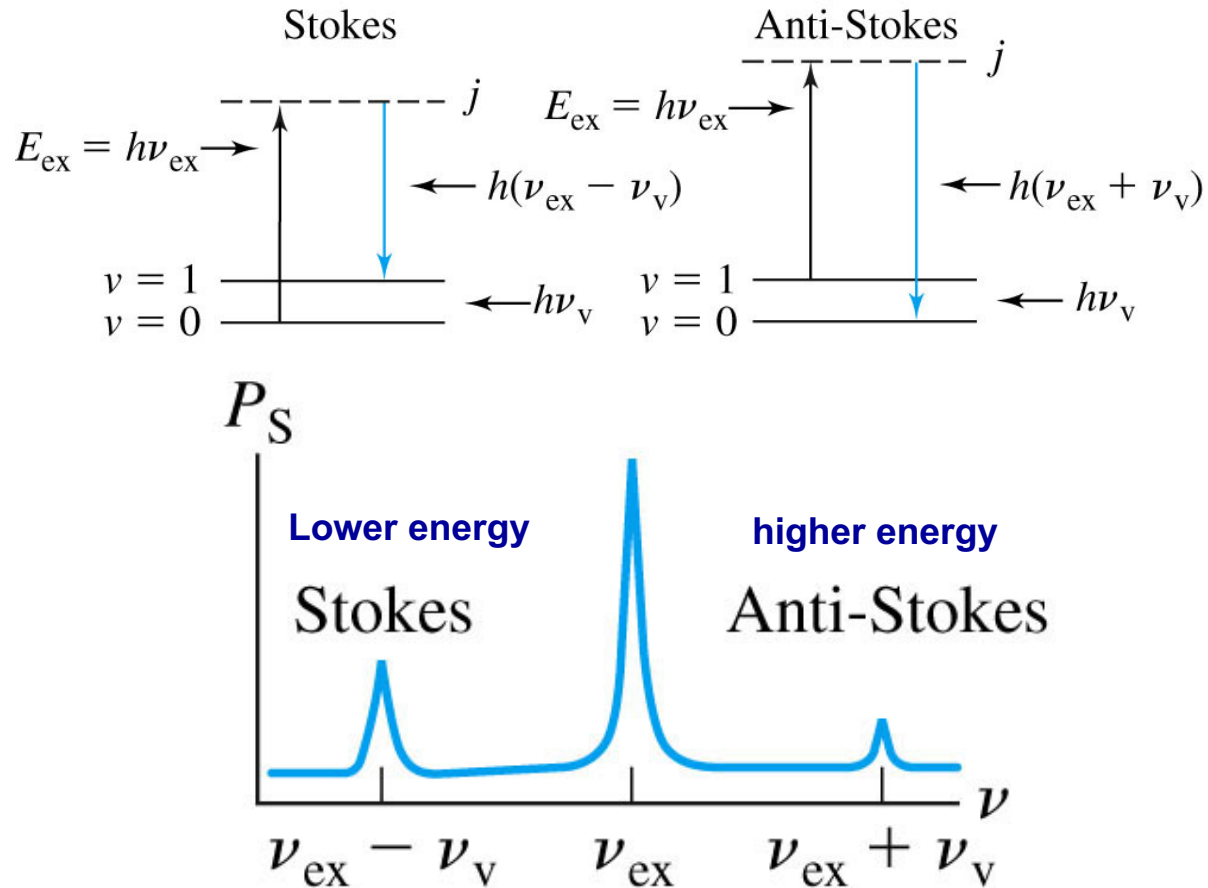
Elástico: colisão entre o fóton e a molécula resulta em mesma energia

Inelástico: colisão entre o fóton e a molécula resulta em alteração da energia



$\pm\Delta E$ – energia do primeiro nível vibracional do estado fundamental – IR vibration absorbance

- ∴ os deslocamentos da frequência Raman e da frequência de absorção no IV são idênticos.



Probabilidade de emissão

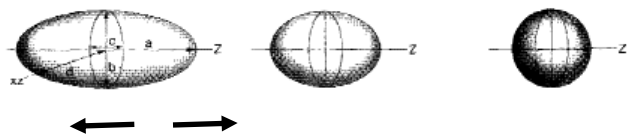
Raleigh scattering \gg Stokes \gg anti-Stokes

Intensidade das linhas Raman são da ordem de 0.001% da intensidade do feixe incidente

3.) Vibrações Raman ativas:

- necessita de uma mudança na polarizabilidade da molécula durante a vibração

- polarizabilidade relacionada à distribuição da nuvem eletrônica



(Capacidade de deformação)

exemplo:



IV inativa

Raman ativa



IV ativa

Raman inativa

IV & Raman são complementares. Existem casos onde as vibrações IV & Raman são ativas (ex. SO_2 – molécula não linear)

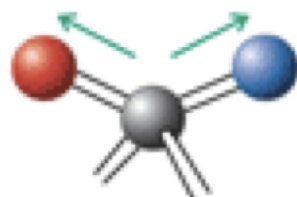
Em geral:

IV tende a enfatizar grupos funcionais polares (R-OH , C=O , etc.)

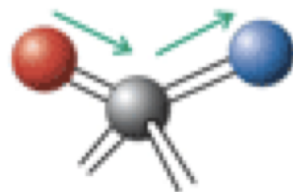
Raman enfatiza esqueleto aromático e de carbono (C=C , $-\text{CH}_2-$, etc.)

- Raman não “detecta” muitos solventes polares comuns, pode ser medido em água – grande vantagem sobre IV

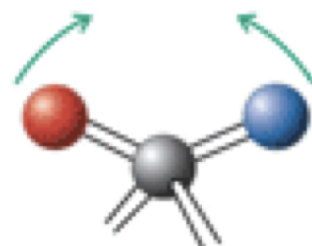
Intervalo de frequências *Raman*: $4000 - 50 \text{ cm}^{-1}$ (Stokes and anti-stokes)



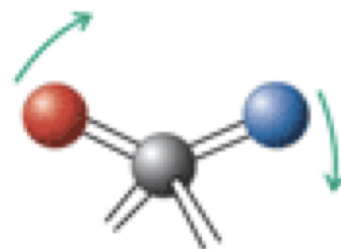
Symmetric stretching vibration (both outside atoms move away from or toward the center)



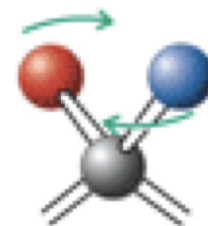
Asymmetric stretching vibration (as one atom moves toward the center, the other moves away)



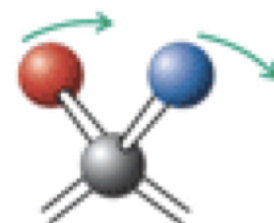
Symmetric bending vibration in a plane (scissoring)



Asymmetric bending vibration in a plane (rocking)



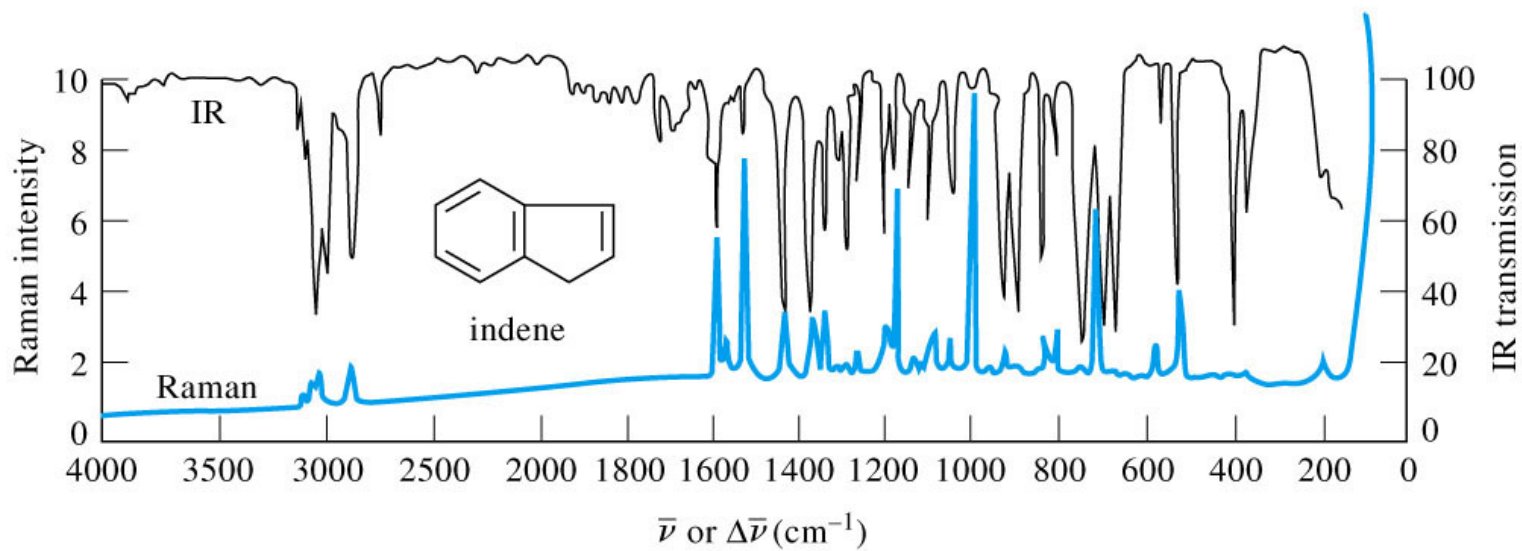
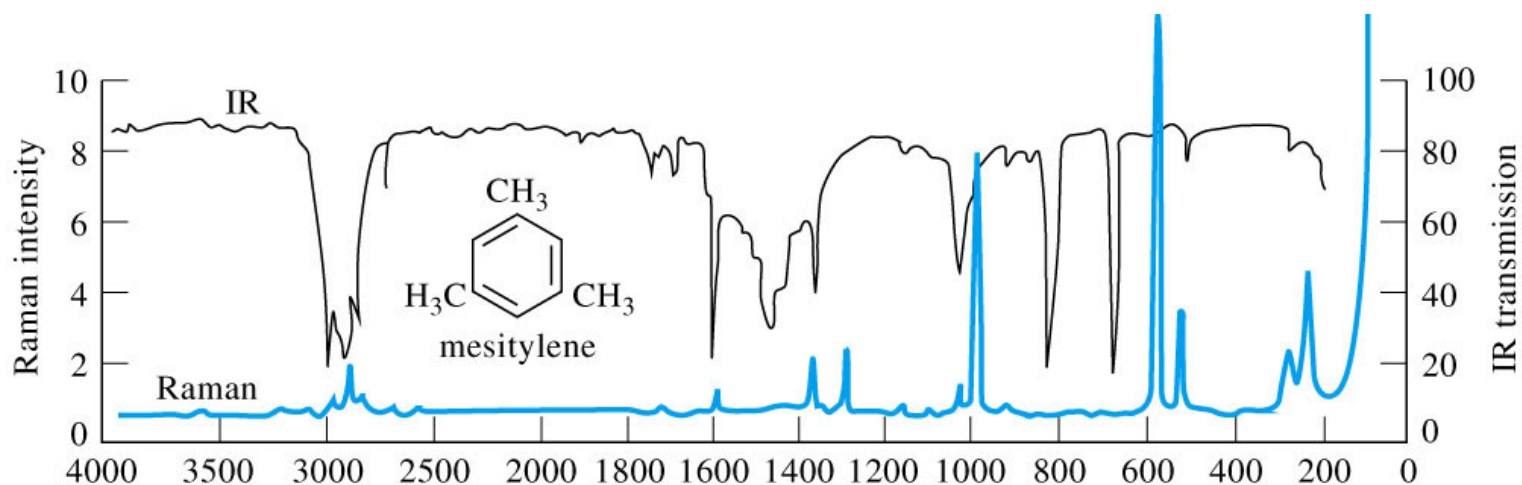
Symmetric bending vibration out of a plane (twisting)



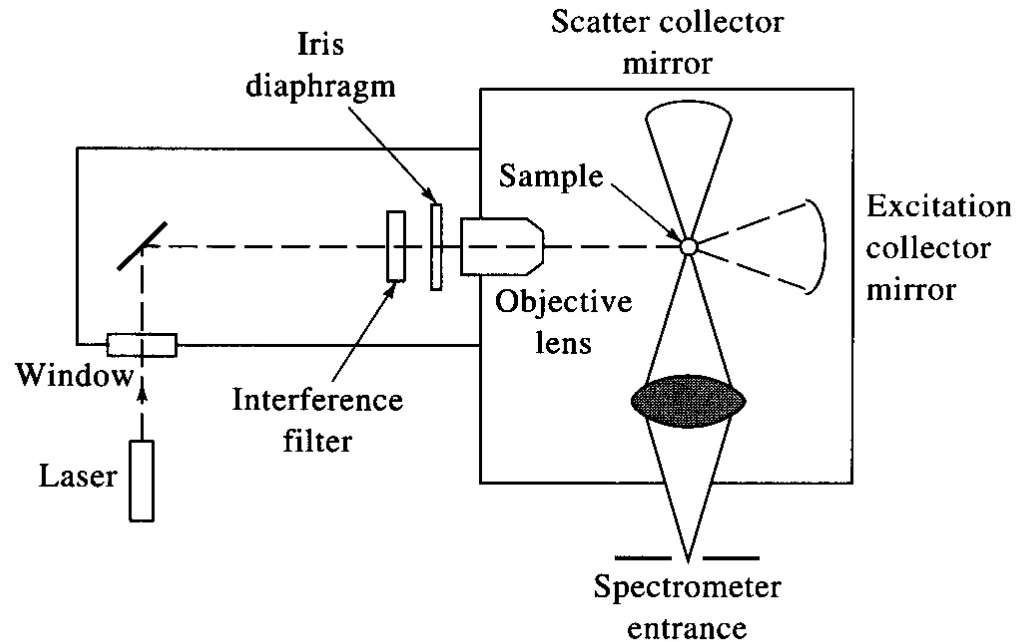
Asymmetric bending vibration out of a plane (wagging)

Figure 3 Examples of vibrational modes in Raman spectroscopy

- comparação entre espectros Raman e IV



4.) Instrumentação:
- Esquema básico



i.) *Fonte de luz:*

- usualmente laser: alta intensidade para obter suficiente razão S/N (signal/noise)
 - Espalhamento Raman é apenas 0.001% da luz incidente
- não precisa ser no IV, uma vez que detecta variações em torno de um pico.
 - Luz visível normalmente apresenta maior intensidade
 - Possibilita o uso de componentes ópticos e de suporte de vidro/quartzo
 - Detetores UV/Vis (PMTs)

4.) Aplicações:

a) Informação qualitativa

- i. regiões características para diferentes grupos químicos
- ii. correlação frequência Raman/grupo disponível
- iii. amostras em água
- iv. variedade de amostras: orgânica, inorgânica e biológica

b) Informação quantitativa – *pouco usual*

- i. menores problemas técnicos do que IV, menos picos
- ii. interferência da fluorescência
- iii. custo mais alto
- iii. sinal fraco – métodos Raman modificados

1) Espectroscopia Raman ressonante: detecção de 10^{-3} -> 10^{-7} com lasers em comprimento de onda próximo à absorção eletrônica

2) Surface enhanced Raman spectroscopy: amostras colocadas em superfícies metálicas (espessura em nanômetros)

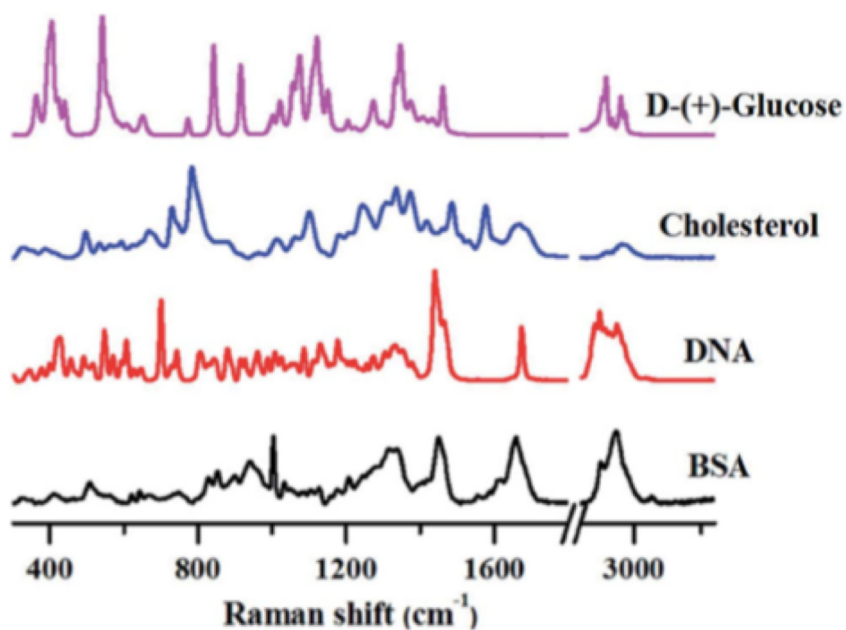


Fig. 1 Distinctive Raman spectra of various biomolecules. All spectra were collected from powdered samples using 785 nm excitation. Spectra were corrected for cosmic rays and baseline corrected prior to analysis.

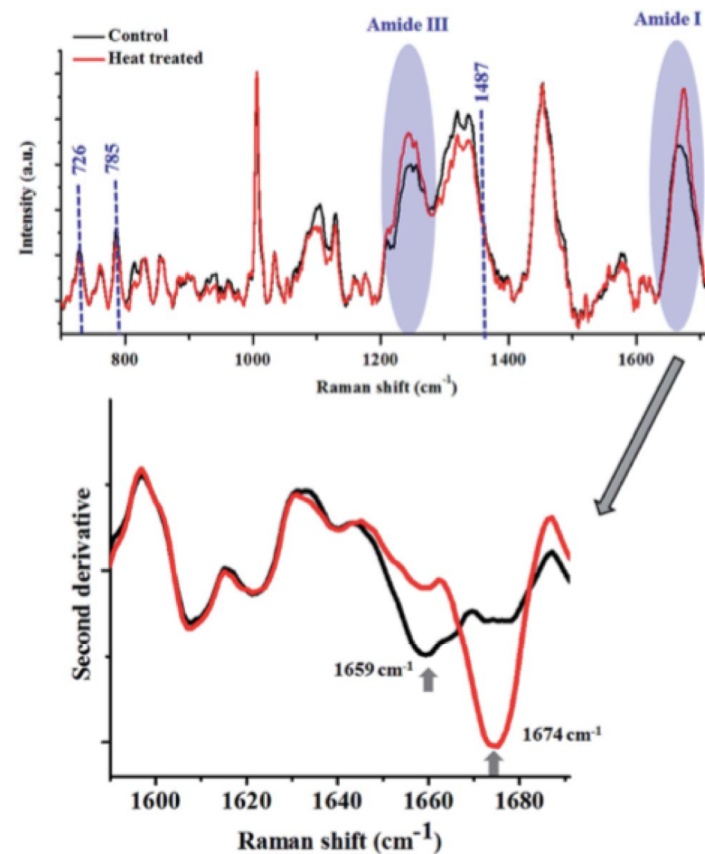


Fig. 5 Raman spectra of bacterial cells before (black) and after (red) heat treatment. *E. coli* WT cells were grown for 8 h in shaking condition at 37 °C and 160 rpm. Bacteria were heat treated at 85 °C for 15 minutes. The shift in the peak maxima of Amide I is shown by the second derivative spectra. All spectra in the top panel were corrected for cosmic rays, background subtracted and vector normalised prior to analysis.

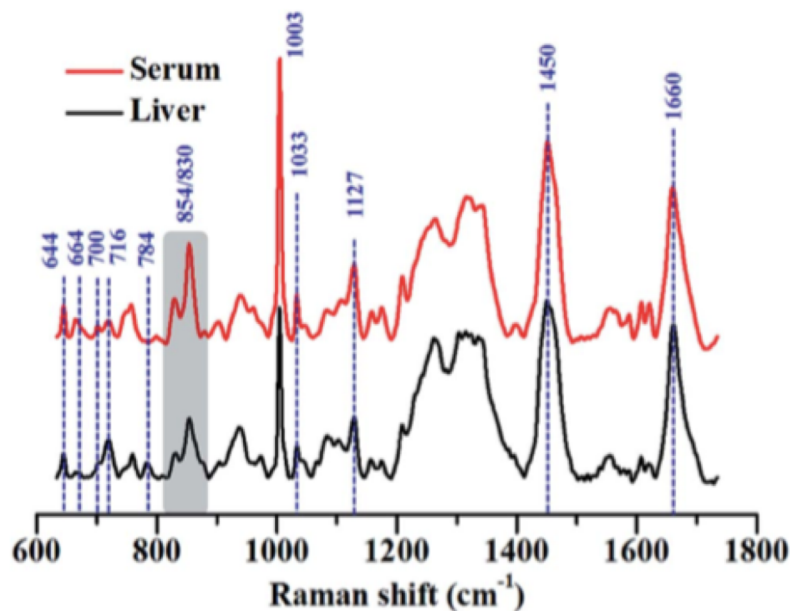
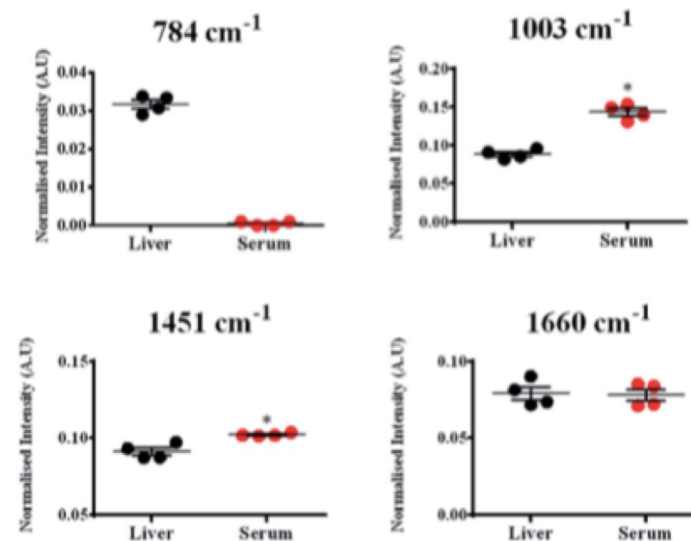


Fig. 3 Average Raman spectra of liver and blood serum isolated from BALB/c mice. The major peaks that were different across the samples have been highlighted using arrows and the different peak ratios that were considered are highlighted using grey boxes. Each spectrum shown is an average of 200 spectra collected from 4 mice. All spectra were baseline corrected, vector normalised and smoothed prior to analysis.

(A) Calculation of intensities



(B) Calculation of intensity ratio

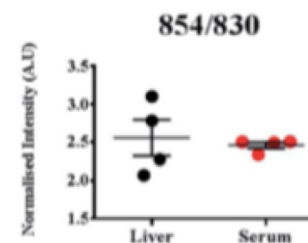
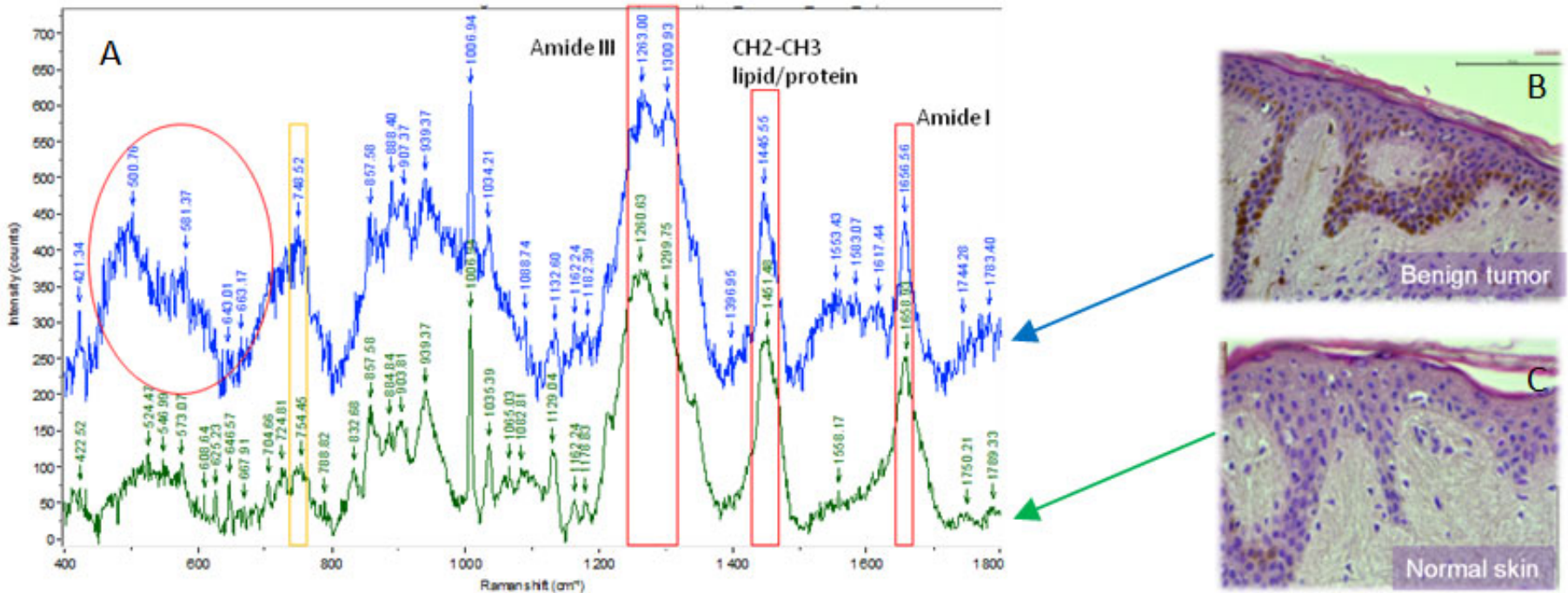
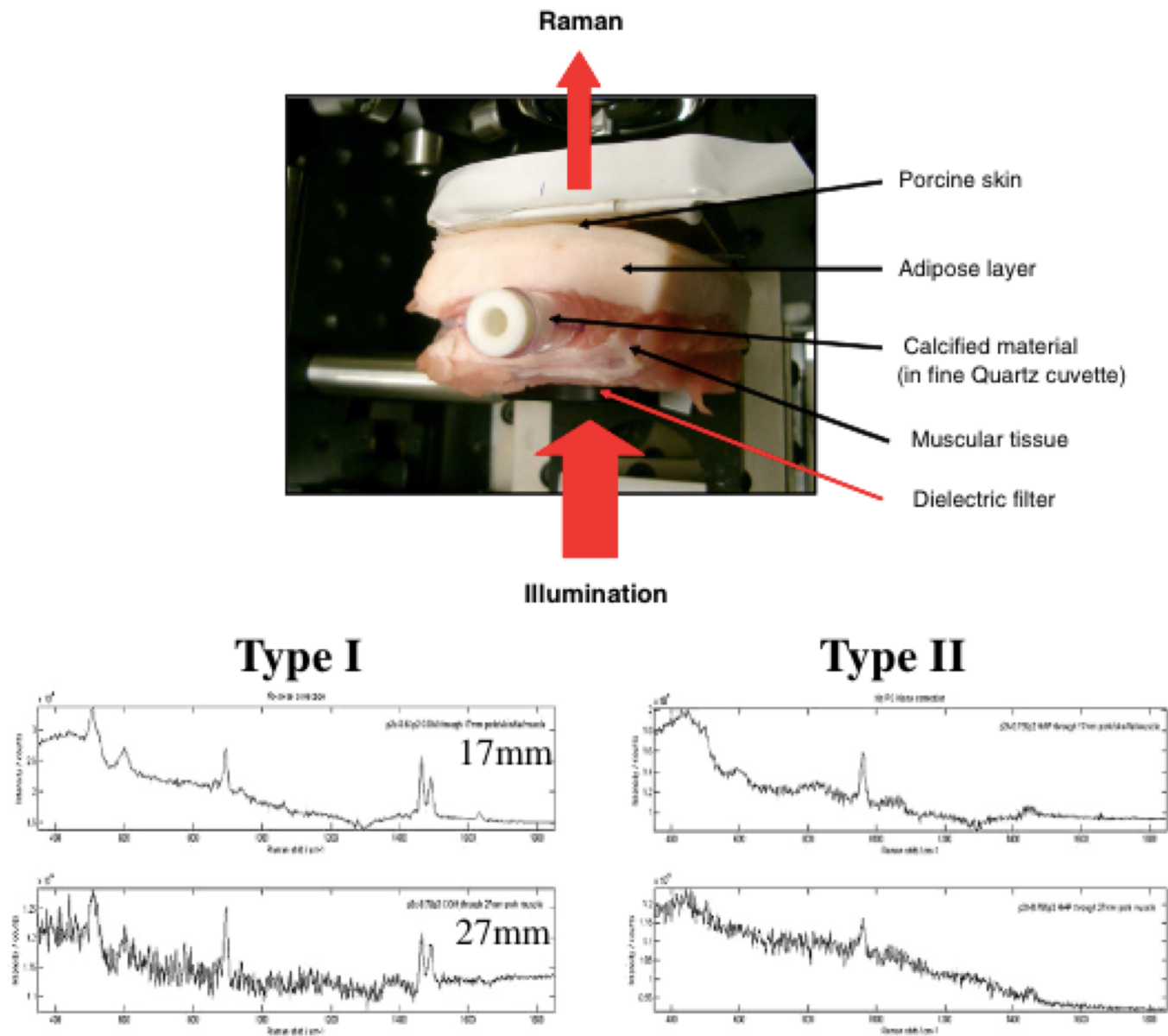


Fig. 4 Calculation of Raman band intensities and intensity ratios pertaining to different biomolecules in liver and serum. Marker bands were chosen for each of the biomolecules; (A) DNA (784 cm⁻¹), proteins and lipids (1003, 1451 and 1660 cm⁻¹). (B) Ratiometric analysis of two Raman bands – 830 and 854 cm⁻¹. Each circle in the figure represents an individual mouse. Spectra obtained from a total of 4 mice were used for enumeration. Statistical analysis was performed using Mann–Whitney test where * indicates $p < 0.05$.



A: Raman spectra shifted (Fig. A: 400-1800 cm⁻¹) of normal and tumor human epidermis; highlighted the bands of the prominent molecular signatures of the numerous constituents of the epidermis. Baseline correction: polynomial fitting function B and C: Haematoxylin and Eosin staining. Original magnification: 40x



Experimental configuration of TRS demonstration [44] using a pork tissue block to simulate human breast and known calcification standards inserted into the centre of the tissue. Laser illumination was used. Note the clear differences in spectra measured from type I and type II calcifications. Reprinted with permission from Cancer Research.

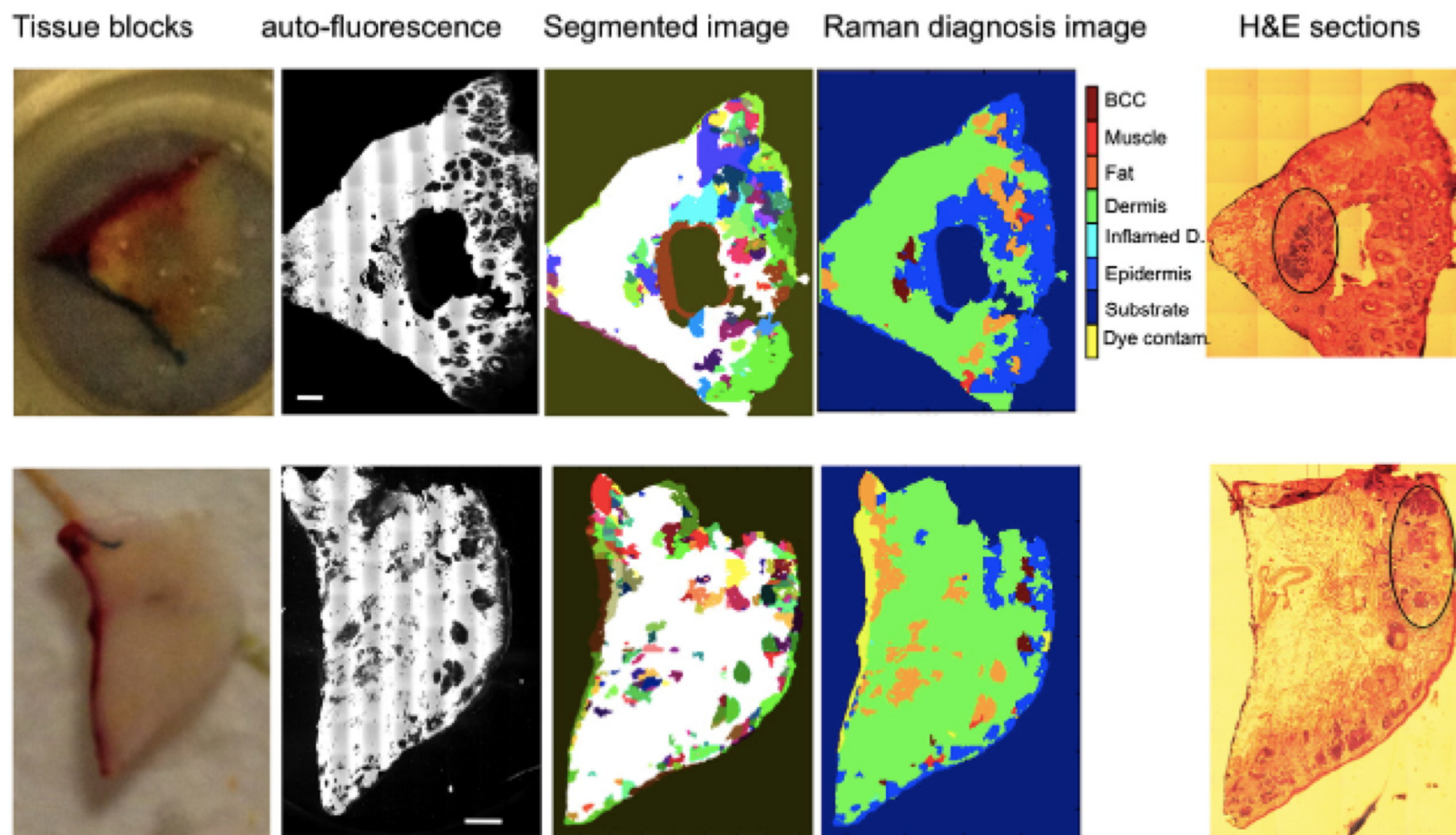


Fig. 4. Diagnosis of basal cell carcinoma of the skin (BCC) by multimodal spectral imaging based on auto-fluorescence imaging and Raman spectroscopy. Scale bar: 2 mm [7]. Reprinted with permission from the National Academy of Sciences of the United States of America.

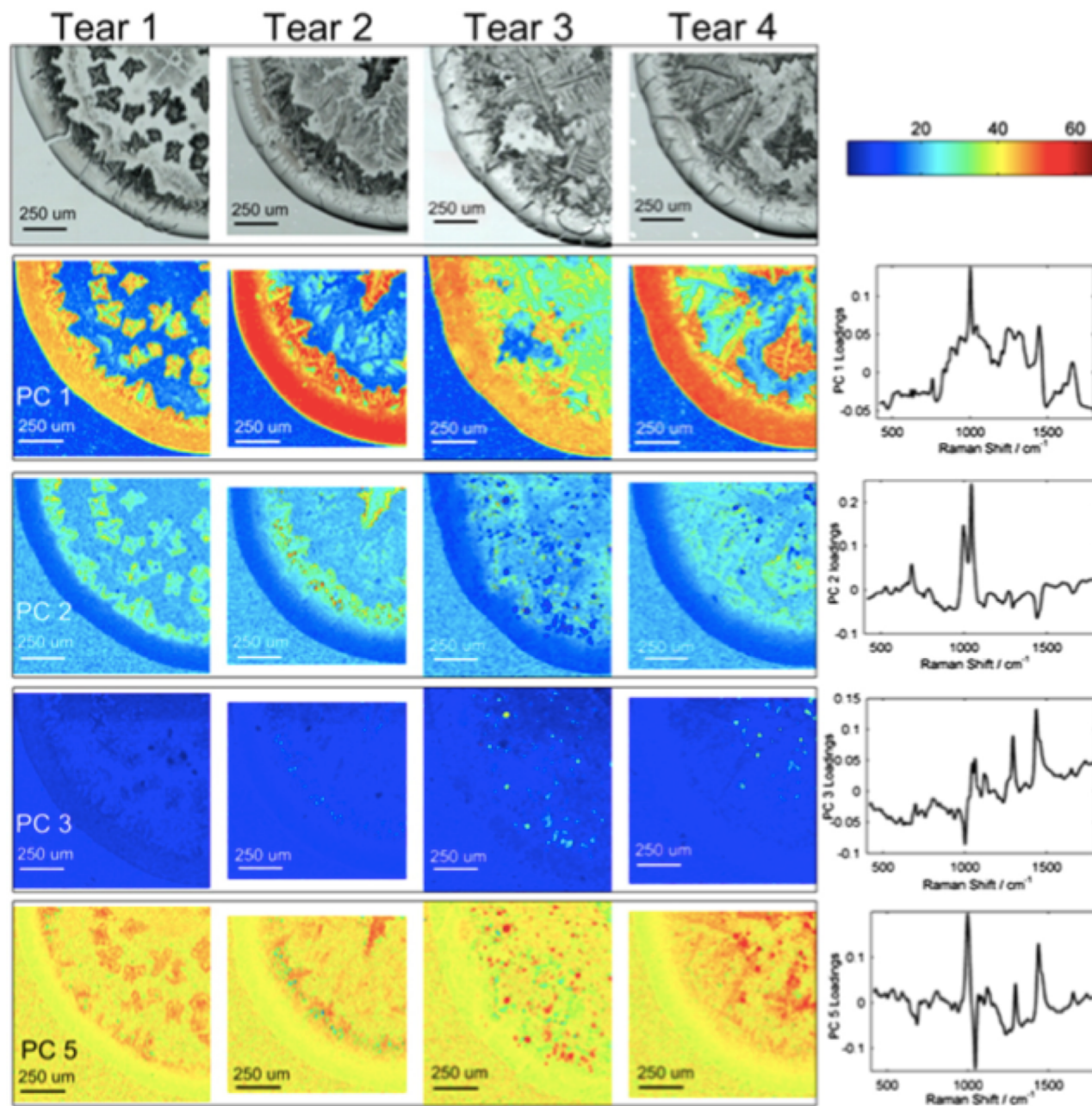


Fig. 5. Four human tear samples drop dried on calcium fluoride substrates and measured with 830 nm Raman microscopy. Top row white light images, subsequent rows are PC score maps and associated loadings (far right). All images are displayed on the same scale [106]. Reprinted with permission from Science Direct.

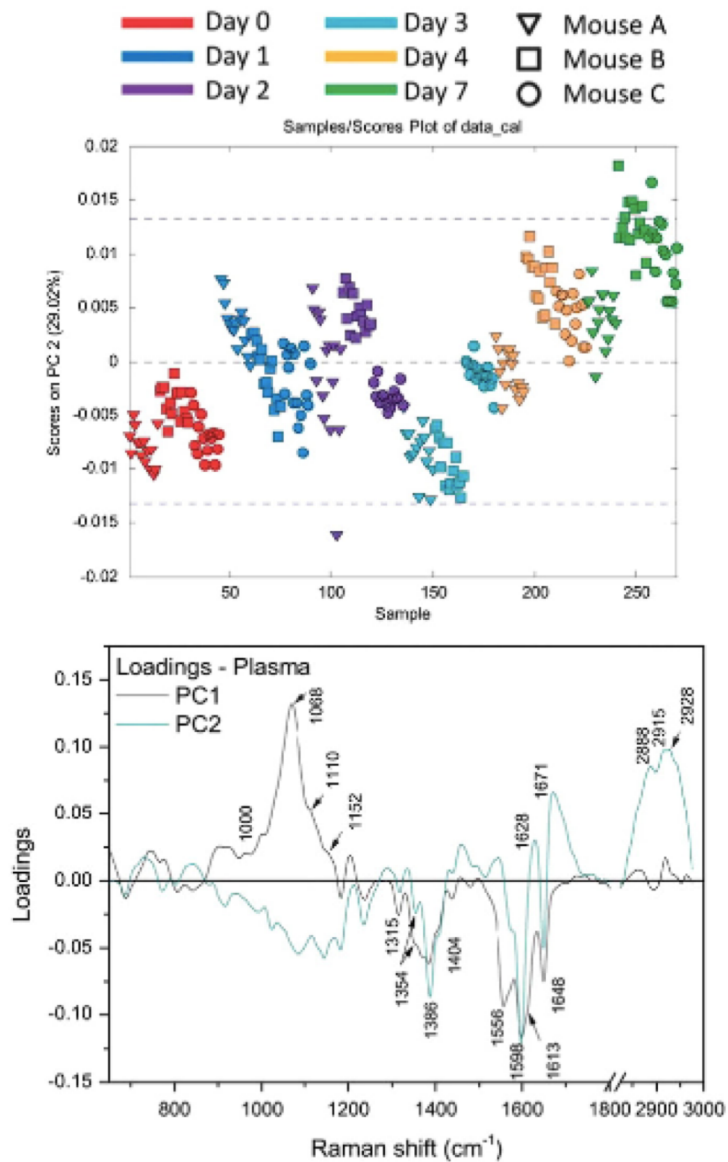


Fig. 6. Principal component analysis of Raman spectra measured for blood plasma from *Plasmodium* infected mice. Samples are as follows: day 0 (red), day 1 (dark blue), day 2 (purple), day 3 (light blue), day 4 (orange) and day 7 (green). Mice A are shown as triangles, mice B as squares and mice C as circles [134]. Reprint with permission from the Royal Society of Chemistry.

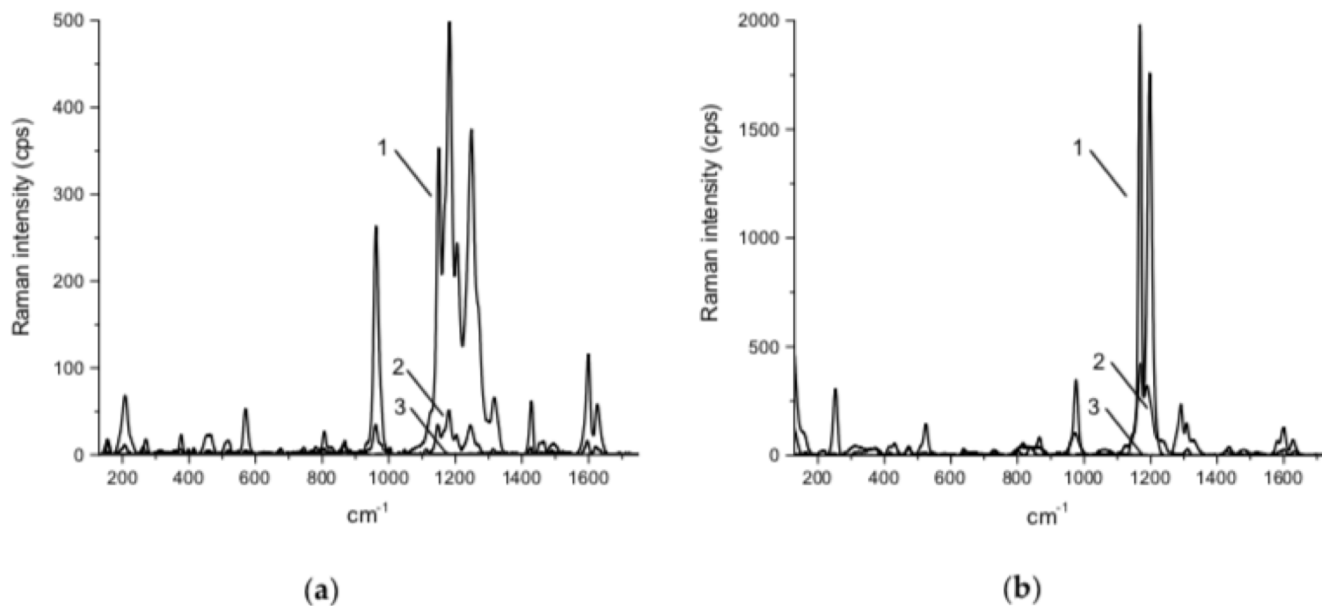


Figure 3. Typical Raman spectra of cream (a) and gel (b) containing curcumin, where 1—Raman spectrum of aggregated curcumin, 2—Raman spectrum of curcumin, dissolved in the base, and 3—Raman spectrum of the base without curcumin.

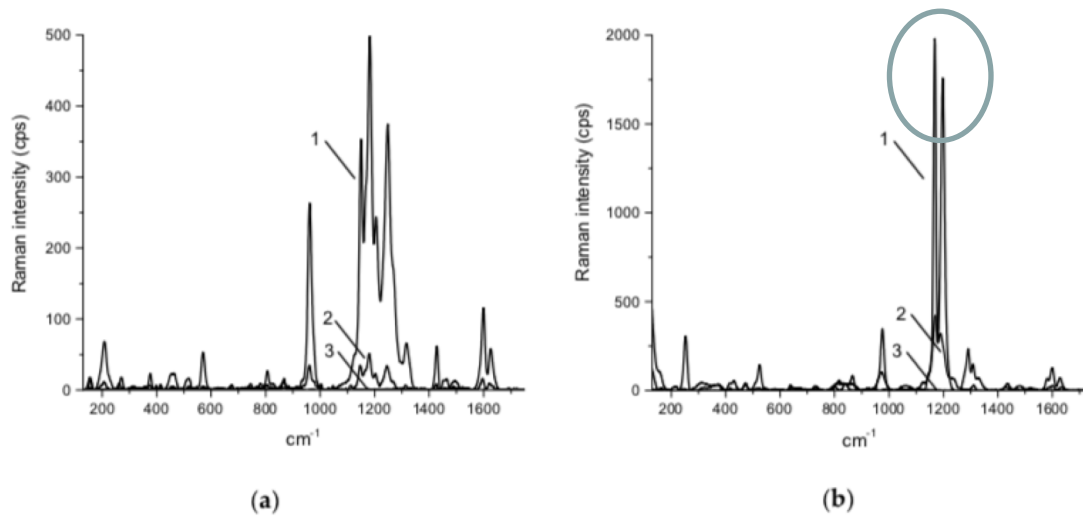


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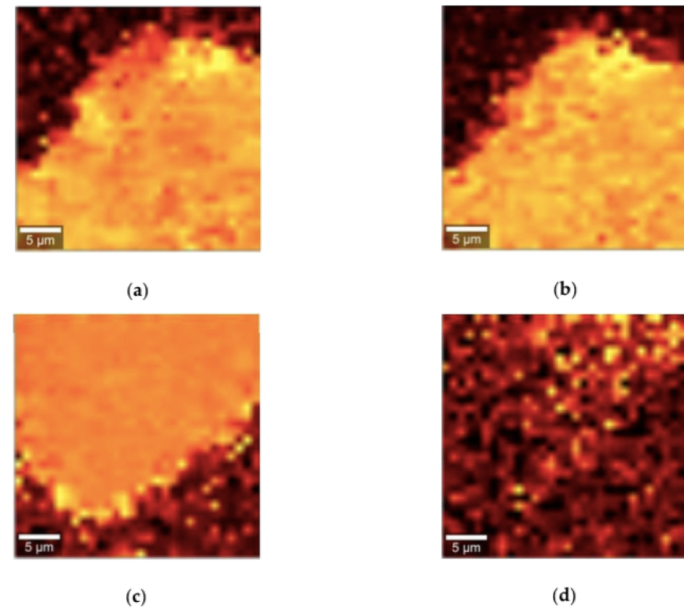


Figure 4. Distribution of Raman peaks at 1182 cm^{-1} and 1252 cm^{-1} in the particles present in the cream ((a,b), respectively) and gel ((c,d), respectively), represented in false colors.