lasers e óptica biomédica

- optical imaging in biomedical sciences -

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mestrado em física médica

introduction

- optical imaging goal
  - contrast enhancement
  - tissue identification (structure, composition)
- scale
  - sub-micrometric (virus, bacteria)
  - micrometric (biological species)
- light
  - reflection
  - transmission
  - fluorescence
- microscope
- scanning microscope
  - confocal
  - laser
- OCT
  - optical coherence tomography
- NSOM
  - near field scanning optical
- TIRF
  - total internal reflection fluorescence

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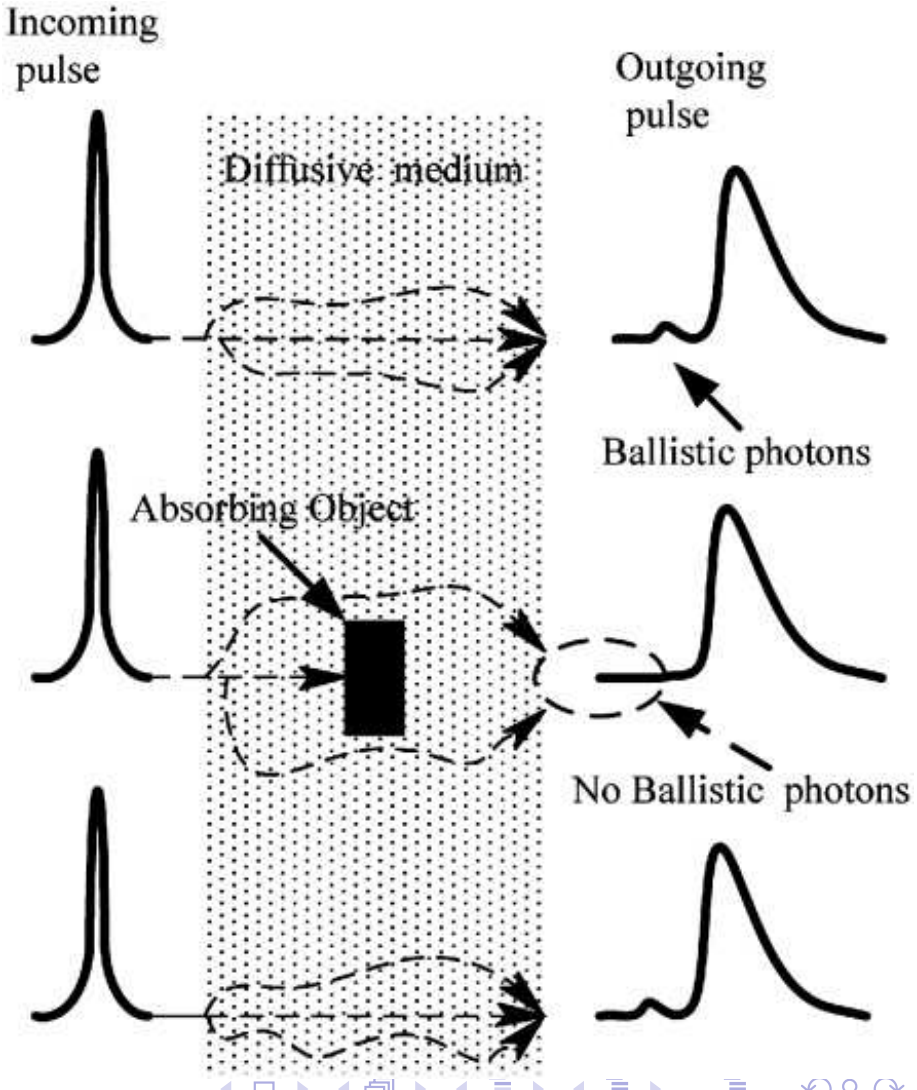
light transmission imaging

- balistic photons
  - structural information
- diffuse light
  - media information
  - to be avoided in optical imaging
    - spatial filtering (*pin-hole*)
    - polarization window
    - time gating
    - spectral techniques



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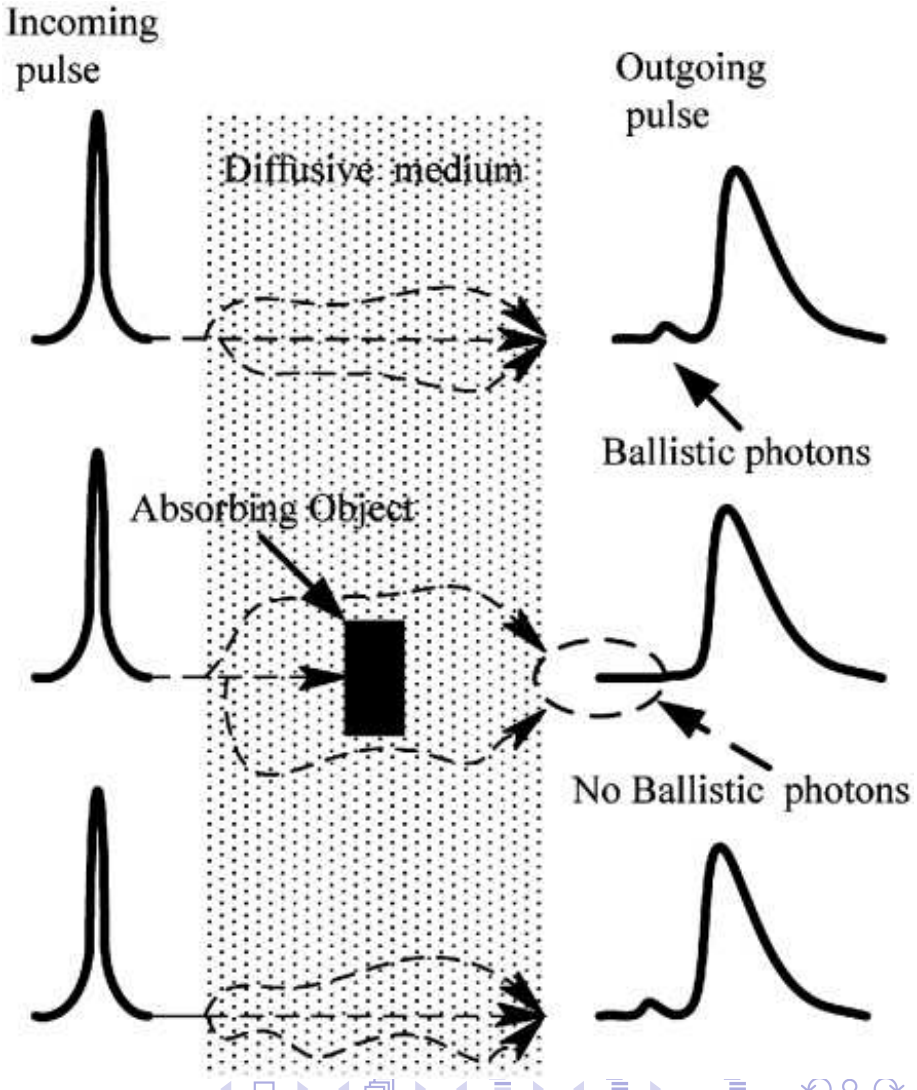


light reflection imaging

- backreflected light from sample
  - coherence (preserves strutural information)
  - diffuse
    - information from the media
    - degrades constrast and SNR
  - optimizing contrast
    - confocal imaging
    - interferometry
    - multiphotonic processes

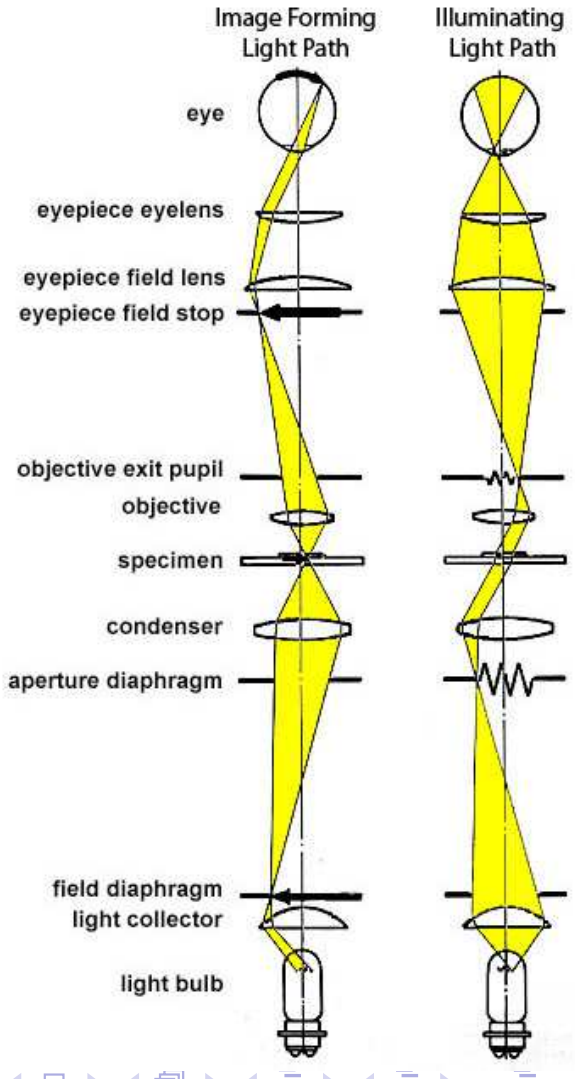
microscópio composto

- magnification:
  - objective: 4 - 100x
  - eye piece (ocular): 8-12x

- focal spot
  - finite tube
    - sample placed outside objective focal spot
  - infinite corrected objective
    - sample over the focal plane
    - additional elements may be positioned inside the microscope tube without disturbing the image

kohler illumination

- aperture diafragm
  - collection of light from sample
- field diafragm
  - (uniform) background illumination and luminosity



primer@micro.magnet.fsu.edu

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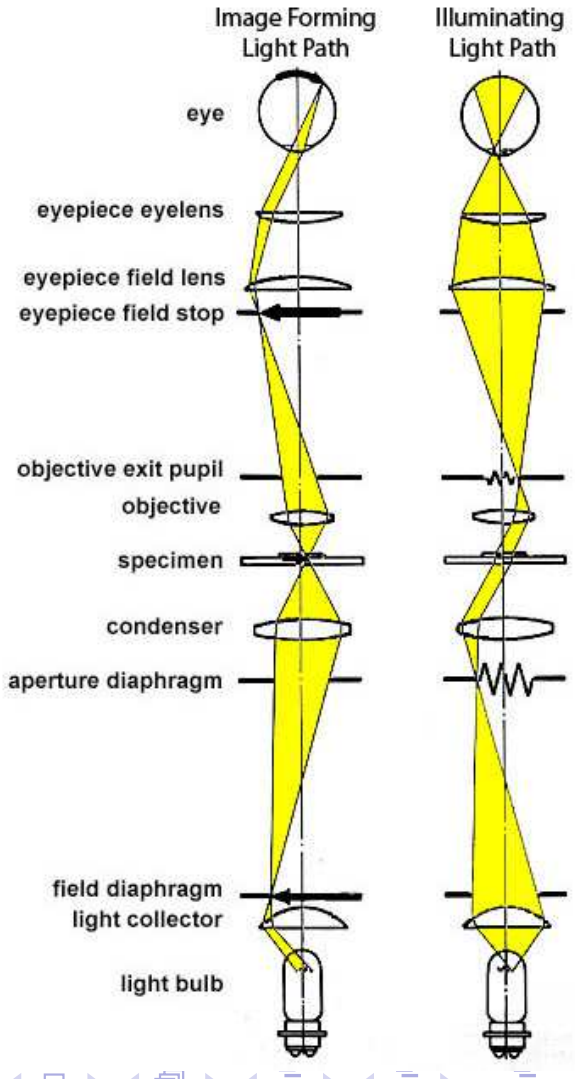

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transverse resolution

- distinguishing adjacent sample points
  - disco de Airy
  - high NA advantage ($NA = n \sin\theta$)

$$\Delta r = 1.22 \frac{\lambda}{NA}$$

- objectives
  - immersion
    - very short working distances
  - image quality (aberrations)
    - chromatic
    - geometrical

confocal

- http://www.olympusfluoview.com/theory/confocalintro.html

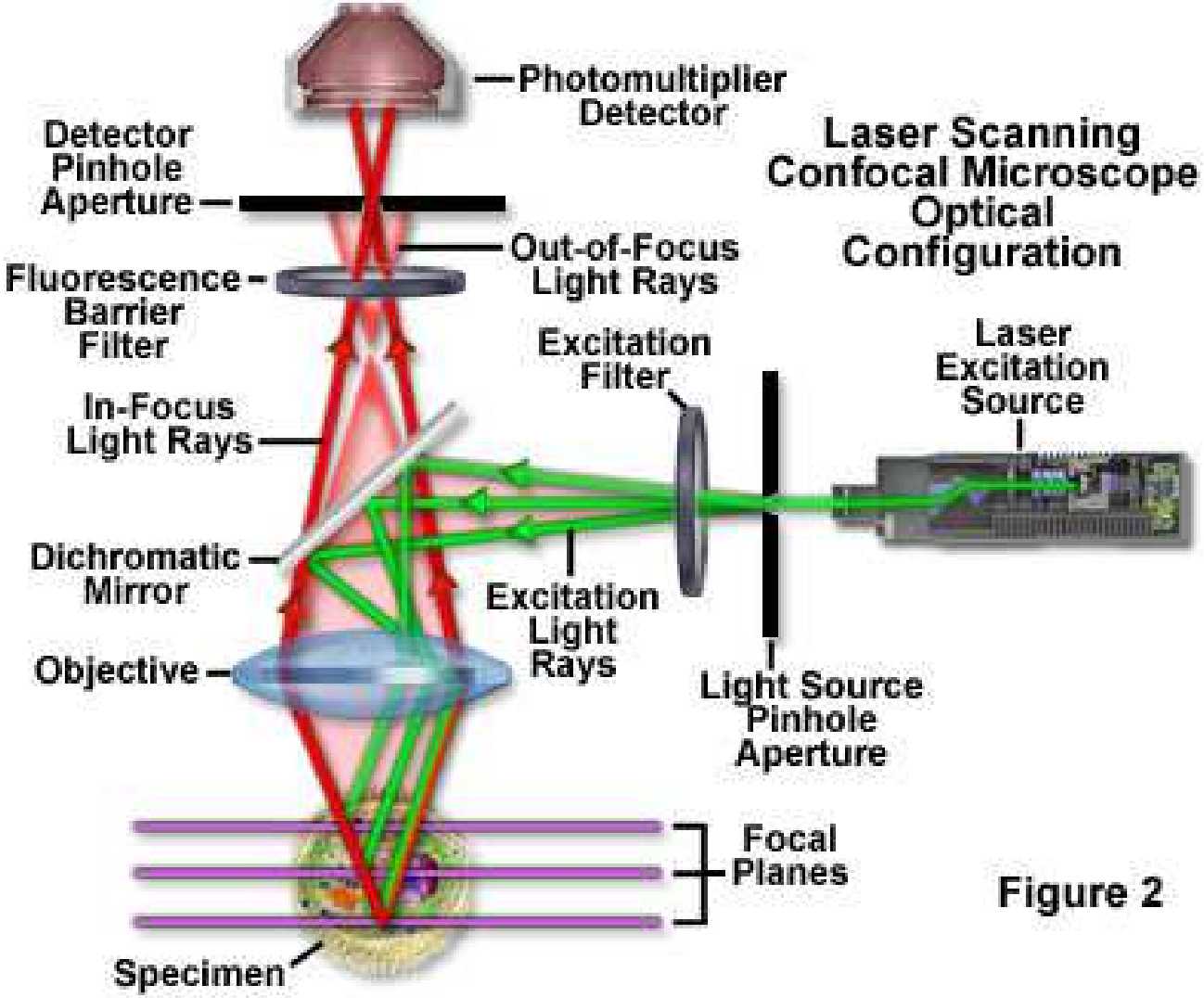


Figure 2

phase constrast microscopy

- **http://www.microscopyu.com/articles/phasecontrast/phasemicroscopy.html**
- thin samples
- light wave phase changes translated into amplitude changes3
- living organisms

fluorescence microscopy

- samples
  - auto-fluorescence
  - optical labels (fluorophores)
- high SNR
- low concentration sensitivity (specificity)
- common configurations: fluorescência + confocal
- http://www.microscopyu.com/articles/fluorescence/fluorescenceintro.html

2-photons microscopy

- two-photon laser scanning miscroscopy
  - http://www.microscopyu.com/articles/fluorescence/multiphoton/multiphotonintro.l
- transition probabilty (excitation) $\propto I^2$
- ultra short pulses, low power
- resolution $\propto \lambda$
  - UV rad for better results, but $\lambda_{fluores} \sim IR$!

TIRF microscopy

- total internal reflection + evanescent field

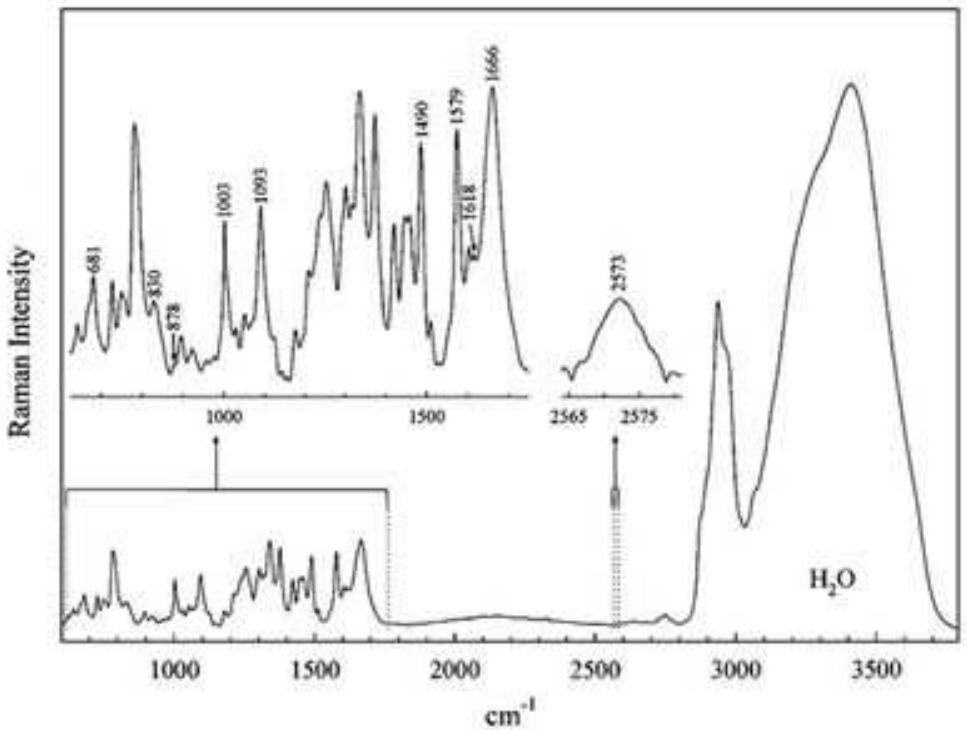
$$E_z = E_0 e^{-\frac{z}{d_p}}$$

$$d_p = \frac{\lambda}{2\pi n_1 \sqrt{\sin^2\theta - \left(\frac{n_2}{n_1}\right)^2}}$$

- low backgroung light
- fluorescence only in focal volume
- http://micro.magnet.fsu.edu/primer/java/tirf/reflect/index.html

Raman effect

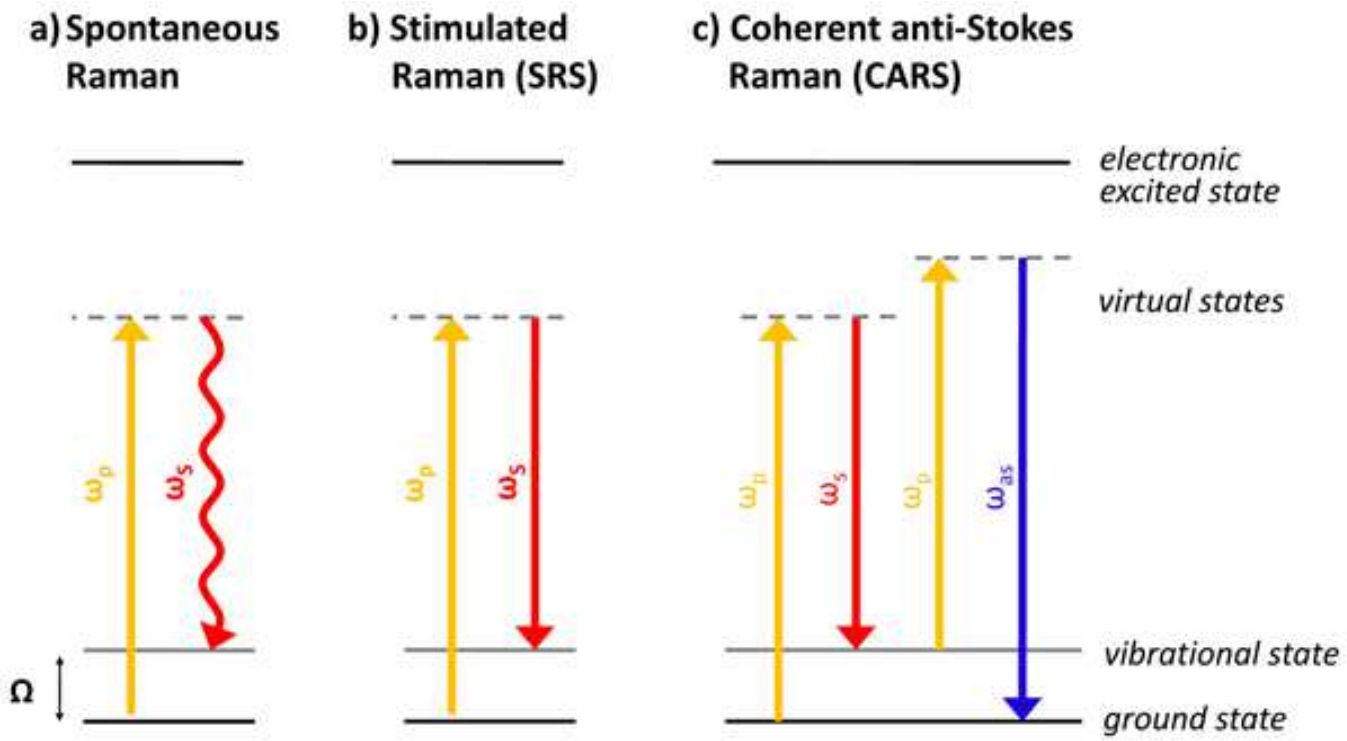
- sensitivity to chemical species without labels
  - Raman spectrum



  - http://bernstein.harvard.edu/research/cars-why.htm

Raman microscopy

- Raman effect: excitation of molecular vibrational states
  - Stokes (reduces frequency, red shift)
  - Stimulated Raman Spectroscopy
    - pumping contains excitation + Stokes frequencies
  - CARS: coherent, anti-stokes, Raman Spectroscopy



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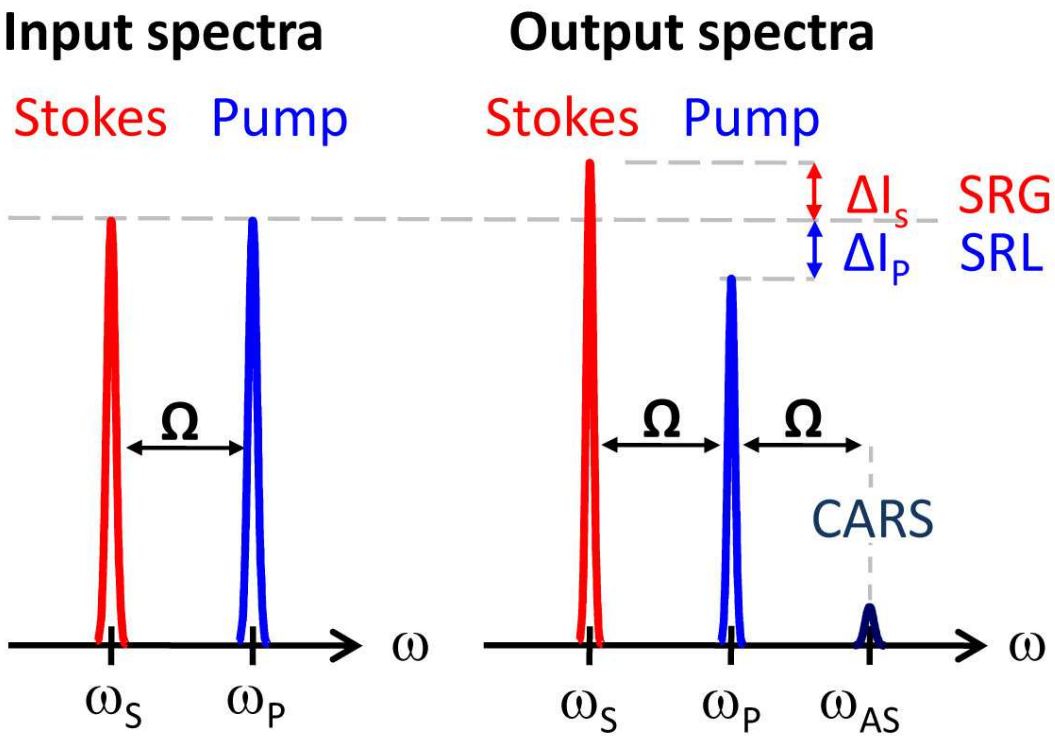

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example: CH2 Raman imaging on fresh mouse skin:Raman Imaging