
# Raman Spectroscopy

17 November 2016

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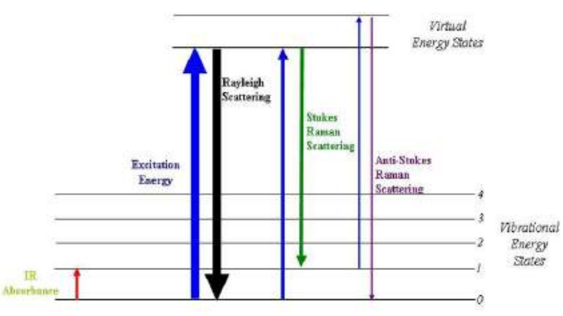
## Outline

- Theory - Raman Scattering
- Instrumental Considerations
- Applications of Raman Spectroscopy: Pigments & Minerals, Paper and Organic materials
- Resonance Phenomena

## Energy diagramme

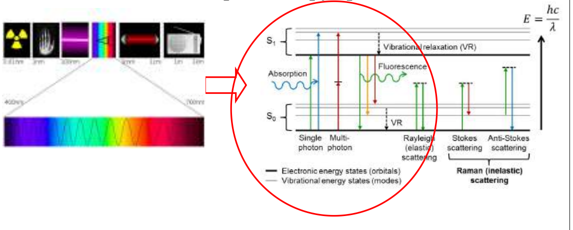



## Complexity of Raman Spectroscopy: Experimental Parameters

- Spectral Resolution (grating, laser)
- Spectral Range
- Calibration
- Number of acquisitions
- Time for each acquisition
- Laser parameters:
  - Wavelength
  - Power density
  - Fluence (energy/ spot size)
- Microscope parameters
  - Magnification, focal distance, aberrations

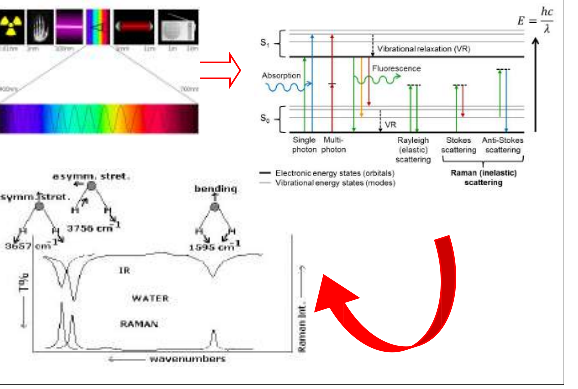

1st Main limitation of Raman Spectroscopy: burning samples

## Raman Spectroscopy:spectrum

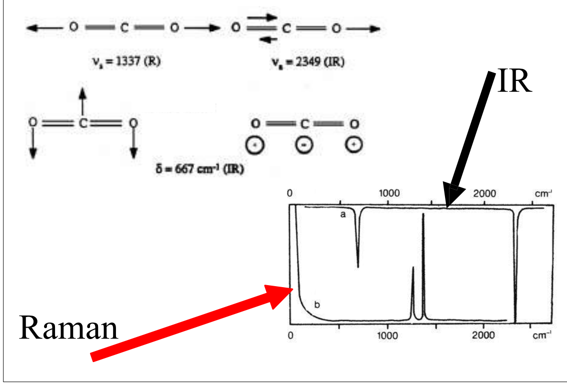



2nd Main limitation of Raman Spectroscopy: luminescence

## Raman Spectroscopy

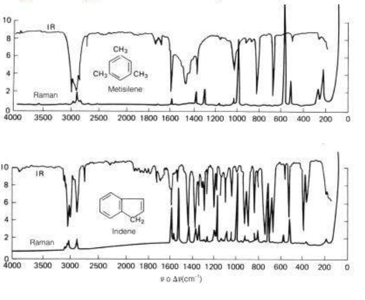



## Raman Spectroscopy vs. FTIR



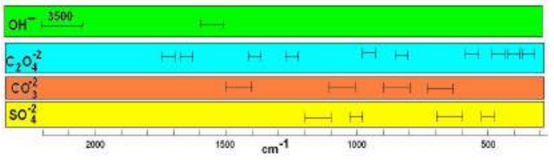

## Comparison of Raman and FTIR spectra

- The Stokes **Raman shift** $\Delta\bar{\nu}$ coincides with **IR absorption lines**
- Some lines are Raman active, other are only IR active
  - The two spectroscopy methods are complementary

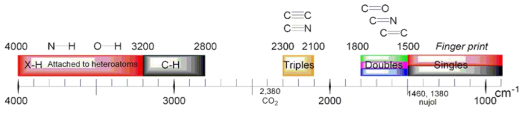


Raman Spectroscopy: fingerprint region

Inorganic molecules

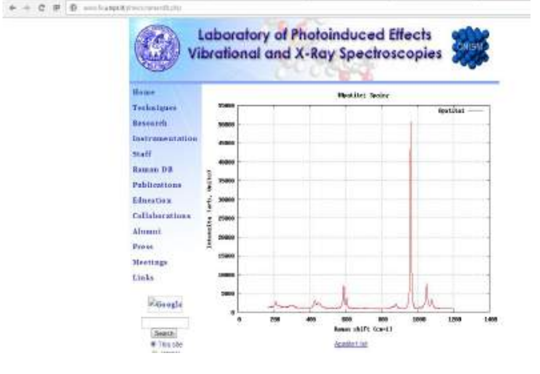



Organic molecules

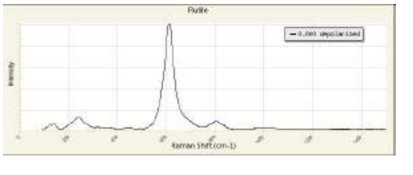


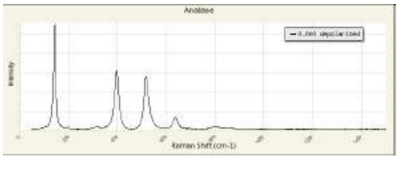

# Databases

- Base de données de spectres Raman (La Société Française de Minéralogie et Cristallographie) (la SFMC)
- Handbook of Minerals Raman Spectra (ENS-Lyon)
- Integrated database of Raman spectra, X-ray diffraction and chemistry data for minerals (RRUFF Project)
- Mineral Raman Database (University of Parma)
- Raman Spectra Database of Minerals and Inorganic Materials (RASMIN) (Ceramics Inst. AIST)
- Raman Spectra of Carbohydrates (Royal Vet. & Agric. Univ.)
- Raman Spectroscopic Library of Natural and Synthetic Pigments (Univ. College London)



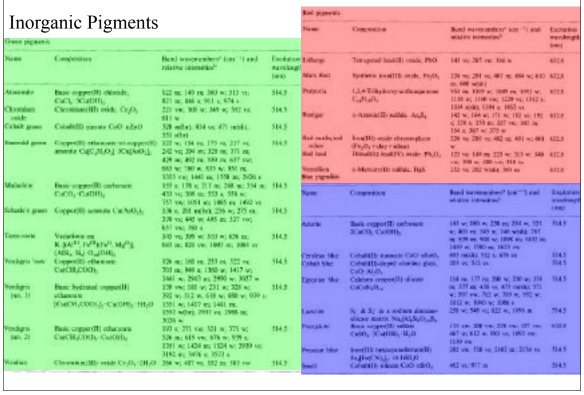

Mineral Polymorphs Raman spectra of Anatase vs Rutile – $TiO_2$



# Raman Spectroscopy: Identification of pigments

Inorganic Pigments



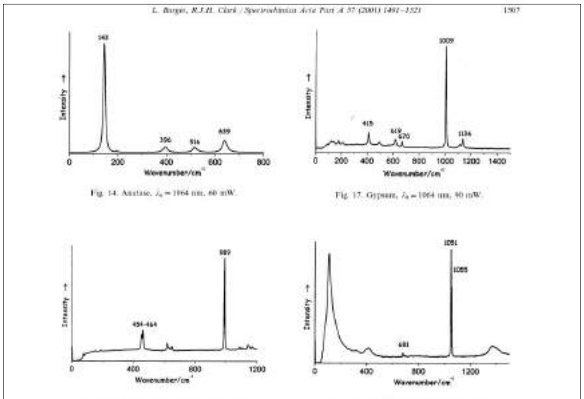



Fig. 14. Anatase, $\lambda_0 = 1064$ nm, 60 mW.

Fig. 17. Gypsum, $\lambda_0 = 1064$ nm, 90 mW.

Fig. 15. Barium white, $\lambda_0 = 1064$ nm, 180 mW.

Fig. 18. Lead white, $\lambda_0 = 1064$ nm, 160 mW.

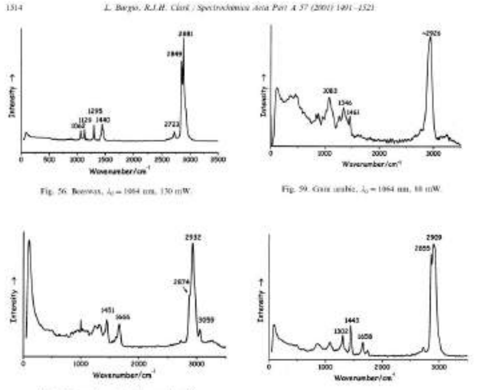



Fig. 56. Beeswax, $\lambda_0 = 1064$ nm, 130 mW.

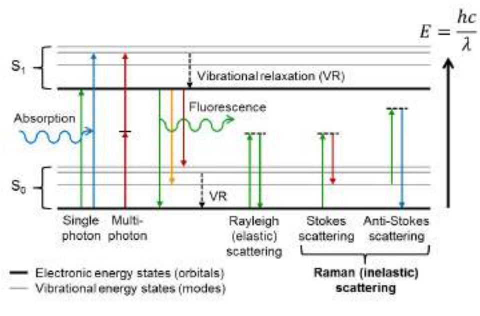
Fig. 59. Gum arabic, $\lambda_0 = 1064$ nm, 80 mW.

Fig. 57. Egg white, $\lambda_0 = 1064$ nm, 130 mW.

Fig. 60. Linseed oil, $\lambda_0 = 1064$ nm, 130 mW.

# What is the largest problem with Raman Detection?

$$E = \frac{hc}{\lambda}$$



# Analysis of Cross-sections

**Raman Analysis:**

- Laser excitation: 514.5 (Ar+) and 785 (Diode) nm
- Objective magnification: 50X (5µm²)
- Spectral resolution: 4-6 cm⁻¹

**Mathematical methods: Subtracted Shift Raman Spectroscopy (SSRS)**
*assume that the fluorescence background can be completely eliminated by subtracting two Raman spectra recorded at two different grating positions*

- easy application
- full automatic spectra reconstruction

Osticioli et al., J. Raman Spec,37, (2006)
Rosi et al., J. Raman Spectrosc. 41, (2010)

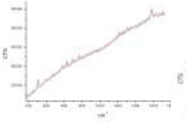
# Subtracted Shift Raman Spectroscopy

Luminescence | Raman Signal

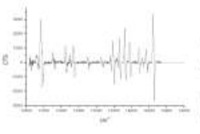
Independent of small variations in frequency ← Excitation wavelength/ grating position → Depends on small variations in frequency

Subtraction

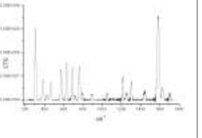
Reconstruction

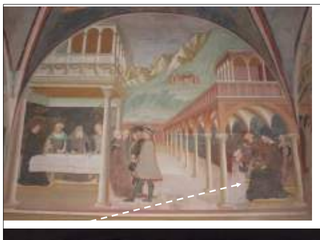


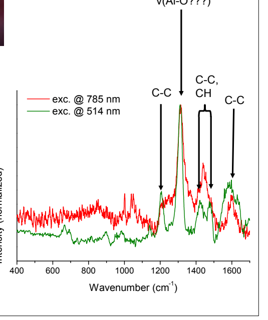
Acquisition



Difference-spectrum

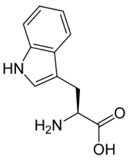


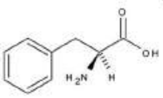


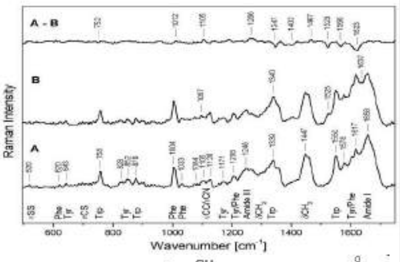



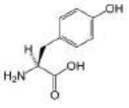

Comelli et al, Journal of Cultural Heritage, 2011

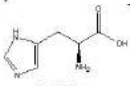
# Raman Spectroscopy of proteins





Histidine

1207.9 (1241.2) amide III C-N str, C-N-H bend
1472.4 (1506.6) amide II C-N str, C-N-H bend
1722.2 (1732.8) amide I C=O str
3582.7 (3582.8) amide A N-H str

# Experimental Parameters

**Renishaw 2000 Micro-Raman spectrometer**

- 785 nm diode laser
- Power 6 mW
- 50x objective for a spot size of 6 µm diameter
- Range: 3500-200 $cm^{-1}$
- 100 s per acquisition, 3 acquisitions: Total time 5 minutes
- no visible damage to samples, or detectable change in spectrum over time

# Raman Spectroscopy of proteins

## Fingerprint Region

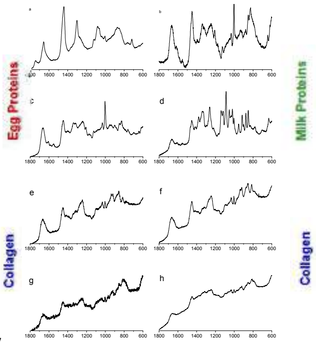
No tryptophan ( 760 $cm^{-1}$) in collagen proteins as in casein, egg white and egg yolk .

Phen and tyr, in all the proteins (850/880 1003, 1030, 1210 and 1605 $cm^{-1}$.

Peak at 1003 $cm^{-1}$ and at 1605 $cm^{-1}$ are especially pronounced in egg white and casein.

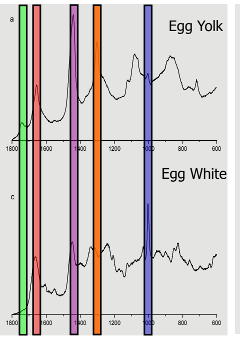
Peaks associated with phosphoproteins in egg white, casein and milk at 954 $cm^{-1}$

Egg yolk, due to the large presence of fatty acid esters,
has a characteristic carbonyl vibration at 1740 $cm^{-1}$



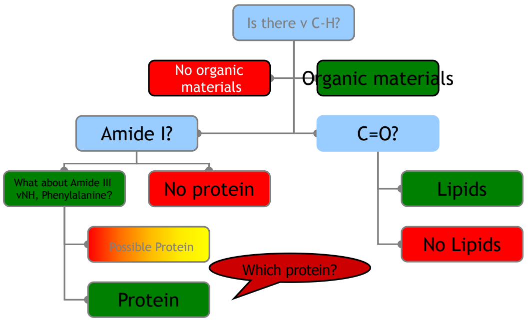

A. Nevin, et al Analytical Chemistry, 2007

# Raman Spectroscopy of proteins



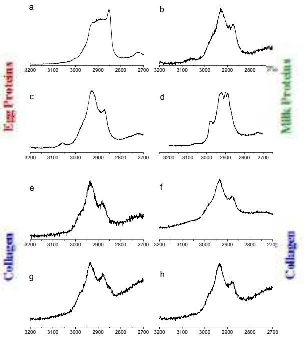

605.07 Amide VI N-H Deformation
718.3 Amide V N-H deformation
766.6 C-H out of plane, Tryptophan
873.49 N-H Tryptophan
1003.3 C-C deformation Phenylalanine
1080.1 C-OH, C-C, C-N Stretching
1125.3 C-OH, Backbone C-N Stretching
1301 C-N-H in plane bending, Amide III C-N stretching
1440.4 CH2 Bending
1656.6 Amide I
1742.1 C=O (only Egg Yolk)
2725.5 C-H stretching
2852.2 Aliphatic C-H stretching

# Raman Spectroscopy of proteins



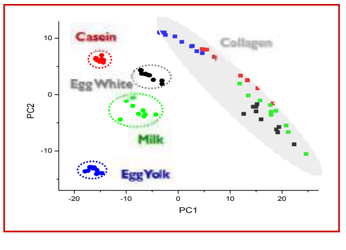

C-H Region

- Differences between Egg Yolk and other Proteins
- Small differences may be confusing with signal noise
- Are C-H different enough to allow differentiation?




# Raman Spectroscopy of proteins

No discrimination among collagen proteins




90% Variation explained with PC1 and PC2

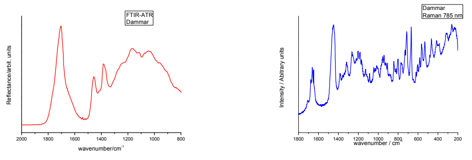
Most variation is with collagen proteins and Milk

PCA of naturally aged binding media

# Media and Varnishes: Various sources

1. Varnishes are often natural products:
2. Tree Resins dispersed in solvents
3. Applied to painted surfaces to: saturate colours, protect the painting
4. Removal of varnishes is sometimes necessary but this may require analysis
5. Ageing of varnish is a significant issue and has been investigated by various groups

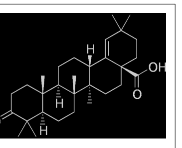
## Varnishes



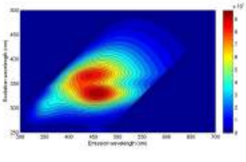

1. **Raman spectra are often well resolved (cf. FTIR) – and can be particularly useful for the analysis of molecular materials**
   - pigments
   - binding media, varnishes, oils, polymers
2. **How is a Raman spectrum recorded?**
   1. Irradiate a molecular sample with a monochromatic source (laser)
   2. Collect scattered irradiation and filter the irradiation with a notch filter
   3. Detect Stokes (or Anti-Stokes) Raman scattering using a monochromator/CCD

Fluorescence of Mastic:

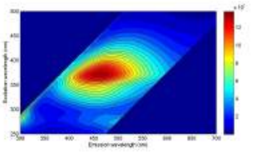
1. Represent as a matrix of excitation vs. emission
2. Measure samples immediately after drying and continue to measure in time
3. Changes in fluorescence are ascribed to rapid oxidation of the varnish



1 week after drying



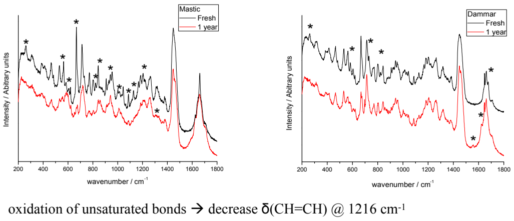
1 month after drying





P. Dietemann, Journal of Cultural Heritage, 2008; A. Nevin, et al. Analytical and Bioanalytical Chemistry, 200; A. Nevin, et al. Talanta, 2009

## Assess molecular changes which




1. oxidation of unsaturated bonds → decrease δ(CH=CH) @ 1216 $cm^{-1}$
2. ring opening → decrease in ν(C-C) ring @ 802 and 772 $cm^{-1}$
3. aromatic ring oxidation → disappearance of δ(C-C) @ 1002 $cm^{-1}$

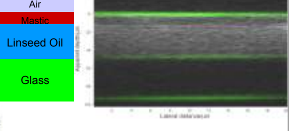
- Accumulation of oxidation products seen with changes of:
- ν(C=C) @ 1624 $cm^{-1}$,
- δ($CH_2$) and δ($CH_3$) @ 1458 $cm^{-1}$
- ν(C-C-OH) @ 858 $cm^{-1}$
- broadening of bands @ 1707 $cm^{-1}$.



A. Nevin, et al. Analytical and Bioanalytical Chemistry, 2009

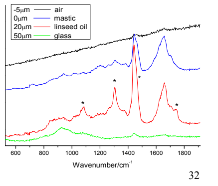
## Confocal Raman - 100X lens and moving stage

- **Results from multiphoton analysis:**
- Detect interfaces in layered samples by measuring changes in fluorescence and 3HG (HSV image)




- **Confocal Raman Spectroscopy:**
- Detect different materials as a function of depth with a resolution of ~ ± 5 μm :

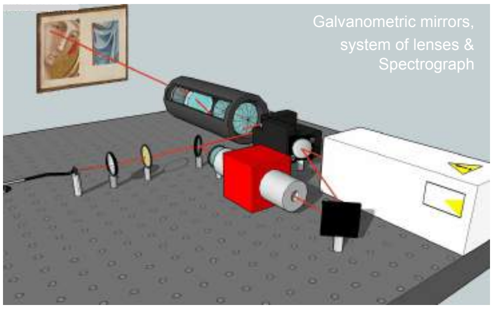
1. Air
2. Mastic
3. Linseed oil
4. Glass substrate






A. Nevin, et al, Applied Physics A, submitted

## Raman scanning device – Diode 785 nm laser

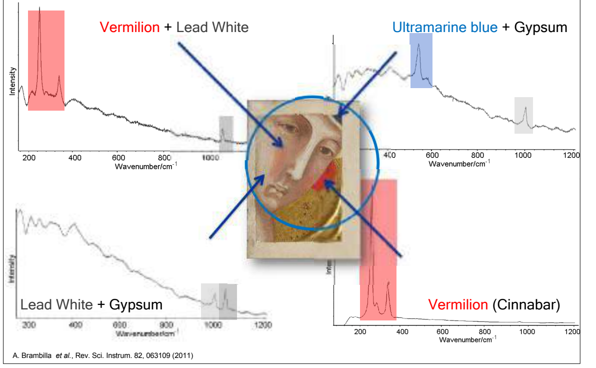



Collect Raman signal from any point of an area of interest (AOI) - 5x5 cm$^2$ @ 20 cm.

A. Brambilla *et al.*, Rev. Sci. Instrum. 82, 063109 (2011)

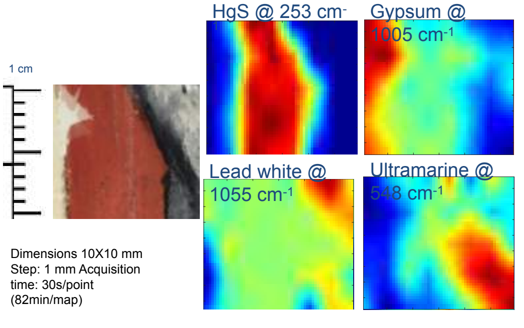
## Non-invasive Raman Analysis : Mapping

Custom analysis (area selected by the user from AOI= 25 cm2 area)




A. Brambilla *et al.*, Rev. Sci. Instrum. 82, 063109 (2011)

## Raman - Mapping




Dimensions 10X10 mm
Step: 1 mm Acquisition time: 30s/point (82min/map)

S. Mosca, et al. Applied Physics A, 2016

## Conclusions

- Raman is particularly useful for microanalysis (picogram sensitivity)
- Complexity due to different laser sources and instruments
- Difficulty in comparing spectra with reference databases in cultural heritage
- Intrinsic limitations
  - Weak signal compared to Luminescence
  - Alteration/burning of material by lasers