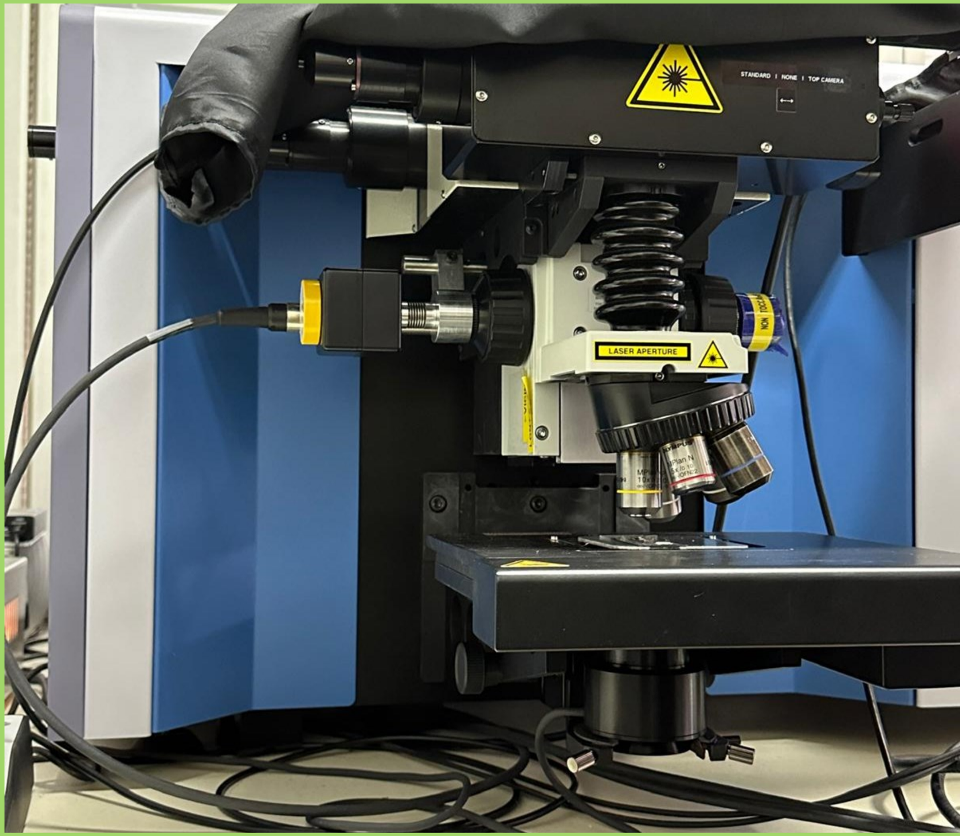
Università degli studi di Modena e Reggio Emilia
*Dipartimento di Scienze della Vita*




# "Un viaggio insieme con la Spettroscopia Raman: i cosmetici antichi, l'amianto, le microplastiche, i composti farmaceutici, le cellule. Scegli il tuo topic e se non c'è ne parliamo insieme!"

**Maria Cristina Gamberini**
**Dep. of Life Science**
**Università di Modena e Reggio Emilia**

Do you know about Raman?

**Think about your research: how can it help me??**

Typical **examples** of where Raman is used today include:

- **Art and archaeology** – characterisation of pigments, ceramics and gemstones
- **Carbon materials** – structure and purity of nanotubes, defect/disorder characterisation
- **Chemistry** – structure, purity, and reaction monitoring
- **Geology** – mineral identification and distribution, fluid inclusions and phase transitions
- **Life sciences** – single cells and tissue, drug interactions, disease diagnosis
- **Pharmaceutics** – content uniformity and component distribution
- **Semiconductors** – purity, alloy composition, intrinsic stress/strain

# Highlights

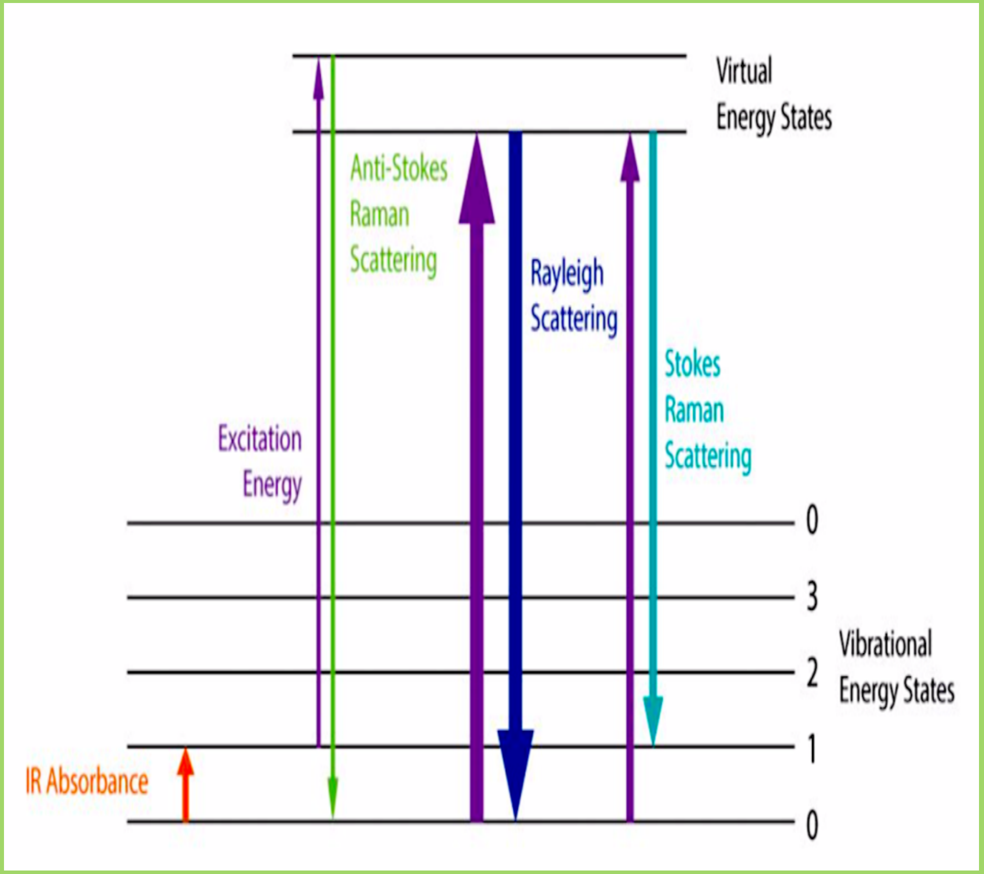
- Raman spectroscopy
- Art and archaeology
- Geology
- Pharmaceutical field
- Raman Cells

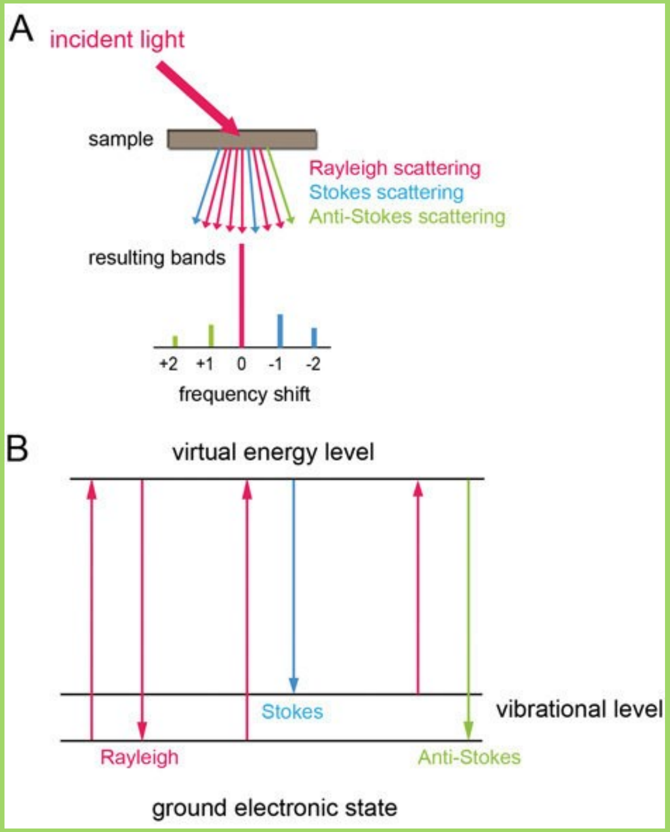
# DIFFERNCES BETWEEN

| Raman | Infrared |
|---|---|
| 1. Scattering of light by vibrating molecules | 1. Absorption of light |
| 2. Due to change in polarisability | 2. Due to change in dipole moment |
| 3. Permanent dipole moment not necessary | 3. Vibrating molecules must posess dipole moment |
| 4. Water as a solvent | 4. Water not solvent due to intense absorption in IR region |
| 5. Tells about covalent nature | 5. Tells about ionic nature |

•

Measures the **inelastic scattering** of photons, when **electromagnetic waves** interact with the sample.
Excited molecules pass to a higher energy state, then relax back: Raman scattering corresponds to the releasing of a different quantity of energy.



# Vibrational Spectroscopy

Vibrational spectroscopy = **MOLECULAR FINGERPRINT**

Each molecular species is uniquely characterised and uniquely identifiable by its spectroscopic fingerprint, called **Raman Spectrum**.

Analysis does **not** require **labels**, and subcellular analysis can be performed in the microscopic mode.

**Vibrational spectroscopy** is the discipline of **analytical science** based on the ***measurement of the vibrations of the bonds within a material***. The combination of vibrations is characteristic of the structure, and the spectrum of vibrational energies can thus be employed as a "fingerprint" which is characteristic of a material or molecular structure, or to monitor changes to it, due to the local environment or external factors (e.g. pressure, temperature, radiation, chemical agents).

- **Label free**, no dyes and toxic waste products
- **Non-destructive**, non-invasive
- High specificity
- **Water can be used** as solvent
- Minimal sample preparation
- Very suitable for biological sample in native state
- Simultaneous detection of macromolecules
- Suitable for chemical analysis, classification and imaging of biological samples

Scattering of light: interacts without RAMAN effetc causing energetic transitions

Laser: **432, 532, 633, 785**
Objectives: **5x, 10x, 50x, 100x, 100LD**
Laser power: 1%, 3%, 5%, 10%, 20%, 50%, 100%
Machine time: Accumulations/Acquisitions
Range used: 200-2000nm

https://youtu.be/b_7M3Vrzo18

# *Spettroscopia RAMAN*

***tecnica NON DISTRUTTIVA***
***utilizzabile anche su***
***Dipinti***
***e***
***Unguentari (contenitori)***

# Campioni Mozia


97

***5th International Conference on the application of Raman spectroscopy in Art and Archaeology***

***14–18 Settembre 2009***
***Bilbao - SPAGNA***

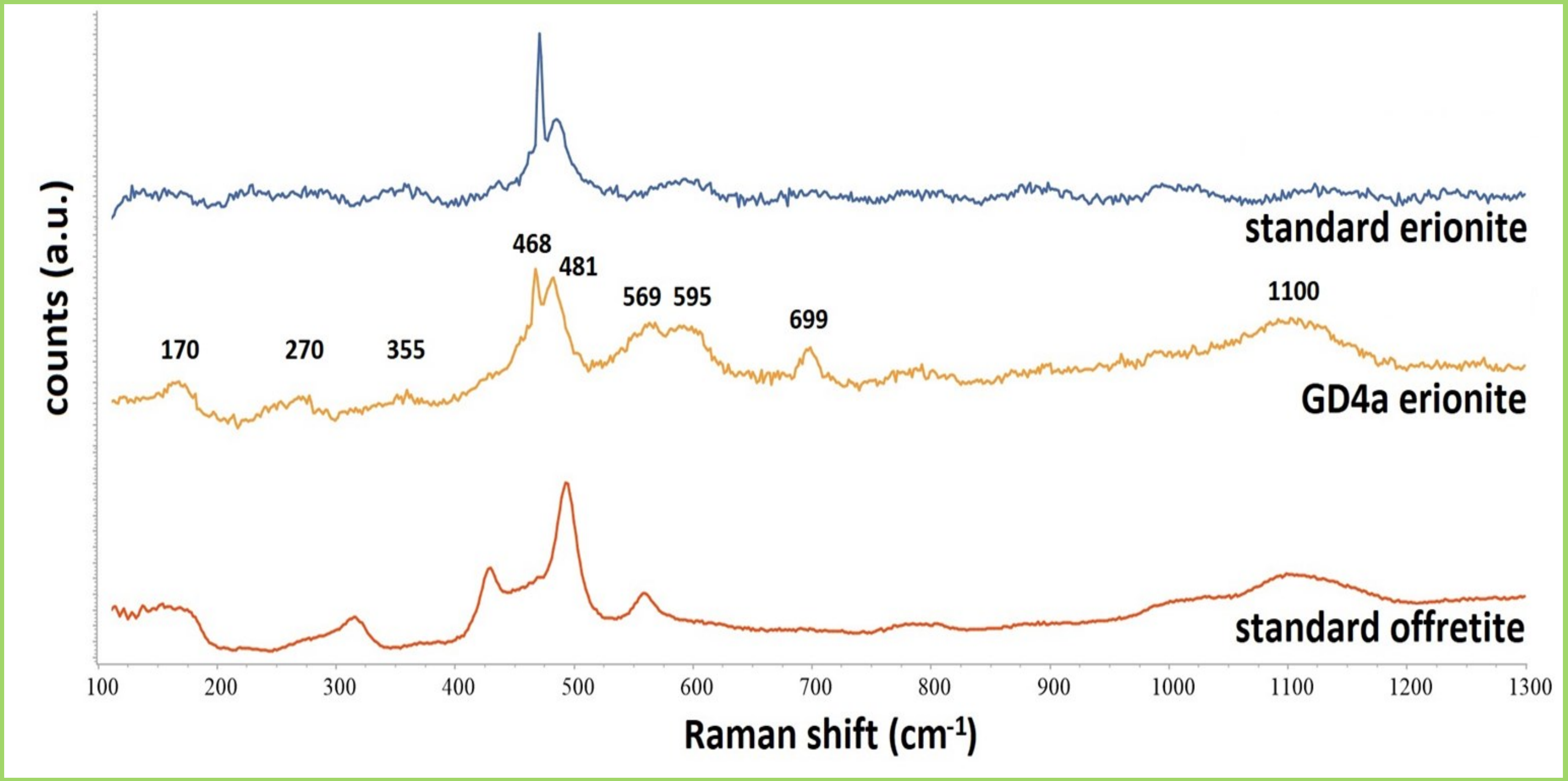
***Direttore del Centro di Ricerca***
***Dr. Philippe Walter***

***Dr. Elsa Van Elslande***

***Centre de Recherche et de Restauration des Musées de France***
***PALAIS DU LOUVRE***


12 Aprile 2011, XIII Settimana della Cultura, SBCA Trapani

**Raman Microscopy for the characterization of geological samples: Erionite** (Gawler Downs, New Zealand)




Sample GD4a was also analysed using micro-Raman with the spectrum reported in Figure (middle curve). The spectrum is compared to that of a **pure erionite-K from Rome, Oregon (USA**; top curve) and a **pure offretite from Saviore (Brescia, Italy**; bottom curve)

Gazzetta ufficiale
dell'Unione europea

IT
Serie L

2024/1441

21.5.2024

**DECISIONE DELEGATA (UE) 2024/1441 DELLA COMMISSIONE**

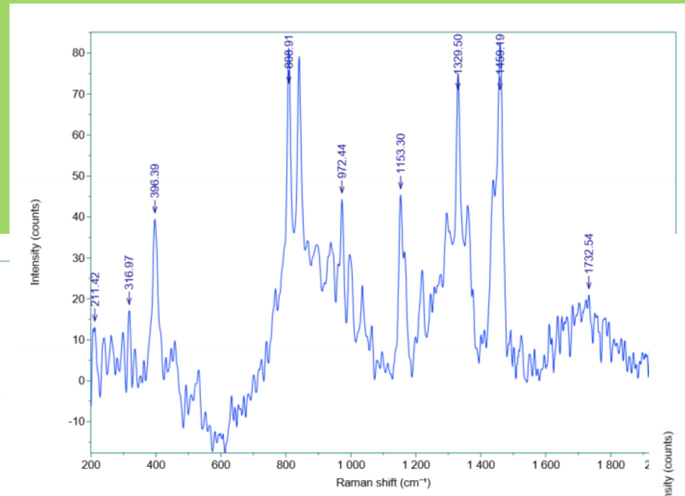
**dell'11 marzo 2024**

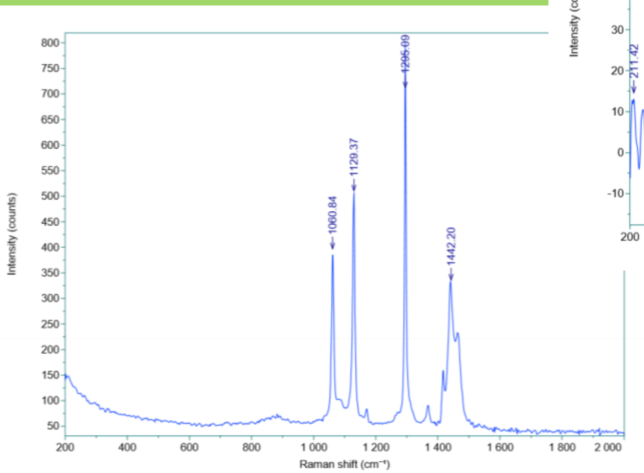
**che integra la direttiva (UE) 2020/2184 del Parlamento europeo e del Consiglio stabilendo una metodologia per misurare le microplastiche nelle acque destinate al consumo umano**

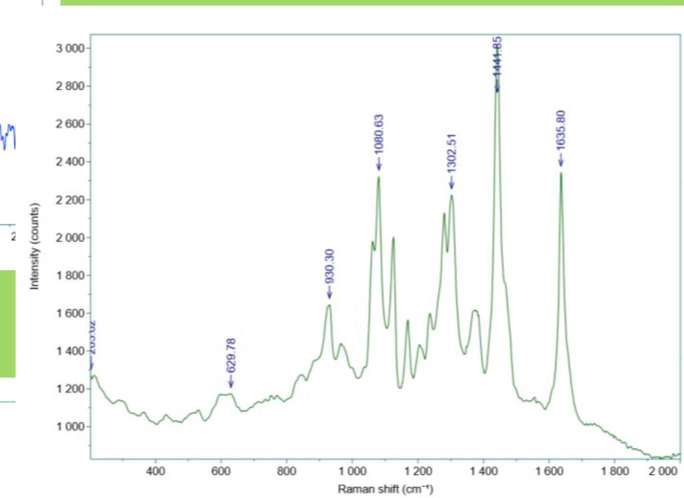
*[notificata con il numero C(2024)1459]*

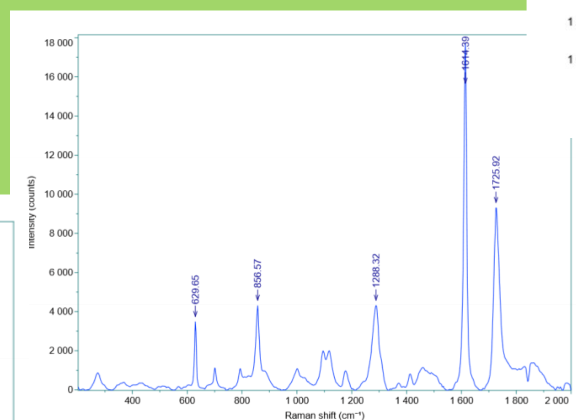
**(Testo rilevante ai fini del SEE)**

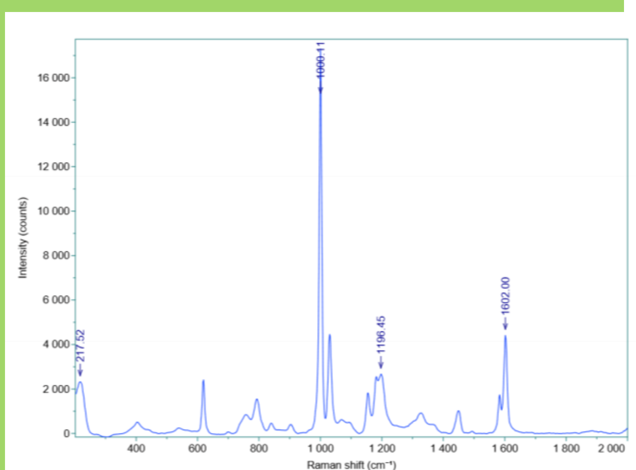
- **Sea degradated plastics**
- **Jellyfish**
- **Ctenophora**
- **Wormdogs**



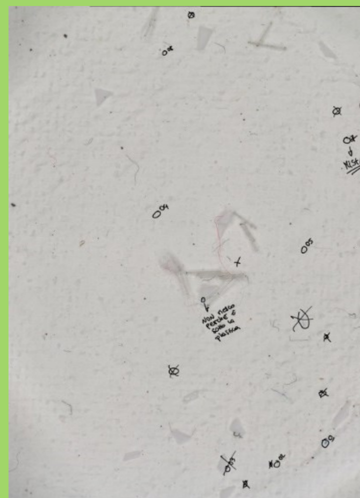
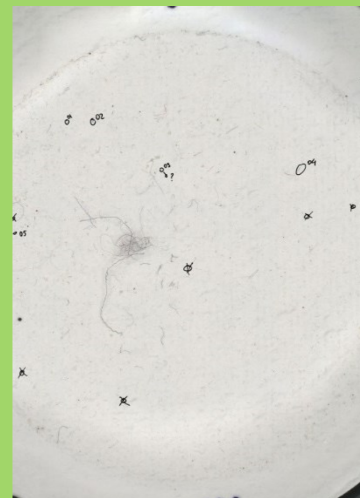



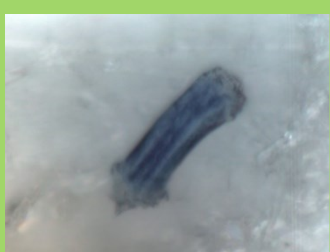
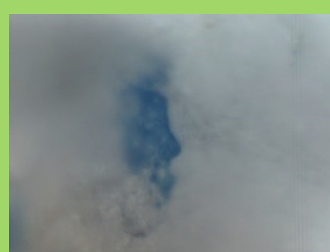
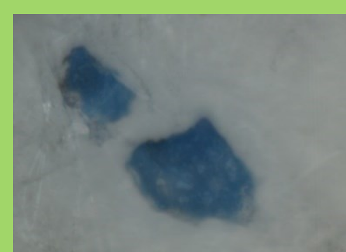

# Microplastics Raman spectra:

# PA, HDPE, PET, PP, PS

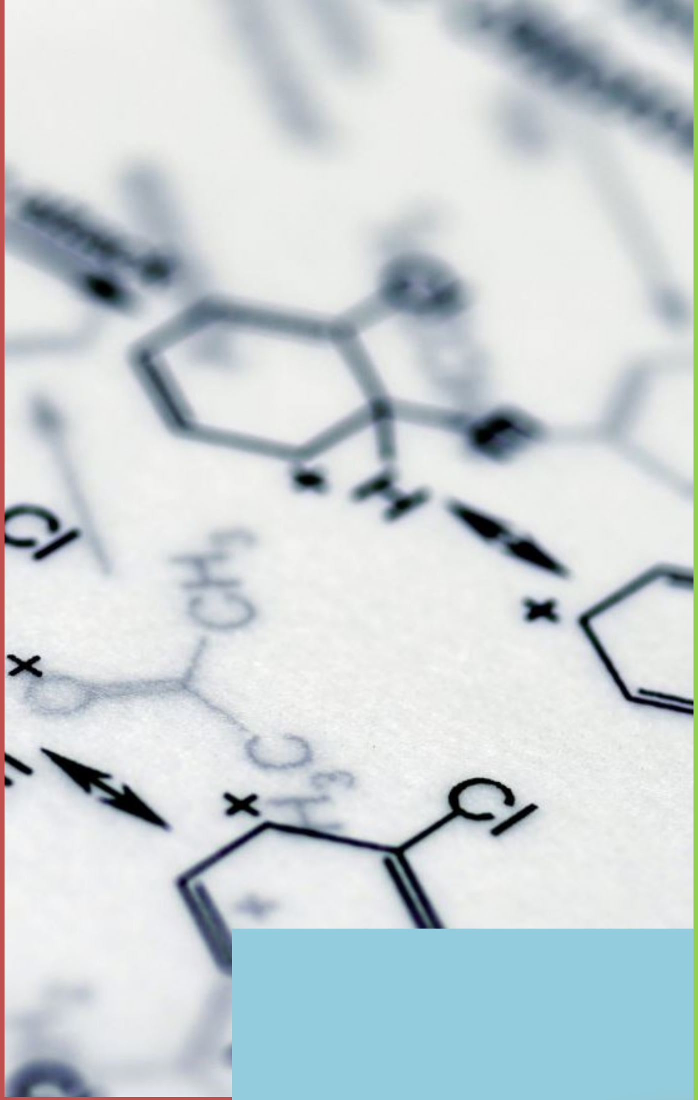



From the optical images, 80 microplastics were found
36 microplastics were Raman analyzed

# n.5 jellyfish's filters

Several individual filter portions were photographed and then joined to, form the complete image.
All the filters were cleaned, washed.



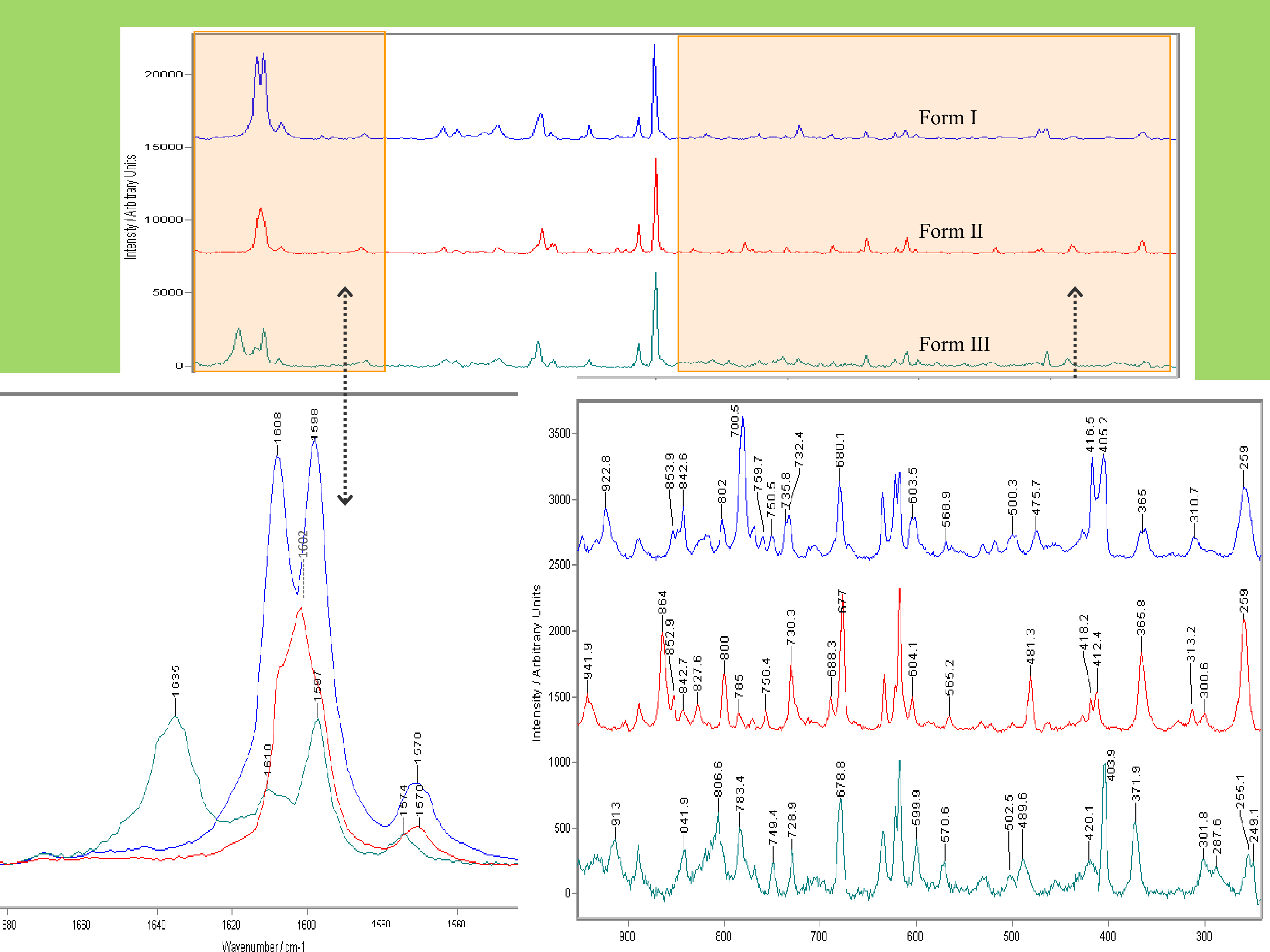
# Pharmaceutical Field
# Polymorph Search

# Analysis of APIs
# Active Pharmaceutical Ingredients

- Exist in a variety of distinct solid forms like:
  - **polymorphs**
  - solvates
  - hydrates
  - salts
  - co-crystals
  - amorphous solids
- Each form displays unique physicochemical properties that can deeply influence:
  - bioavailability
  - manufacturability purification
  - stability
  - other performance characteristics of the drug

***STUDIED*** ➡

- ✓ **Tamoxifen Citrate**
- ✓ **Acetazolamide**
- ✓ **Chloramphenicol**



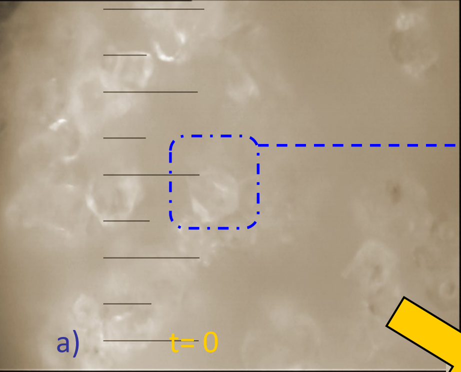
Intensity / Arbitrary Units
20000
15000
10000
5000
0
Form I
Form II
Form III
1608
1598
1602
1635
1597
1610
1570
1574
1570
1680
1660
1640
1620
1600
1580
1560
Wavenumber / cm-1
Intensity / Arbitrary Units
3500
3000
2500
2000
1500
1000
500
0
922.8
853.9
842.6
802
700.5
759.7
750.5
735.8
732.4
680.1
603.5
568.9
500.3
475.7
416.5
405.2
365
310.7
259
941.9
864
852.9
842.7
827.6
800
785
756.4
730.3
688.3
677
604.1
565.2
481.3
418.2
412.4
365.8
313.2
300.6
259
913
841.9
806.6
783.4
749.4
728.9
678.8
599.9
570.6
502.5
489.6
420.1
403.9
371.9
301.8
287.6
255.1
249.1
900
800
700
600
500
400
300

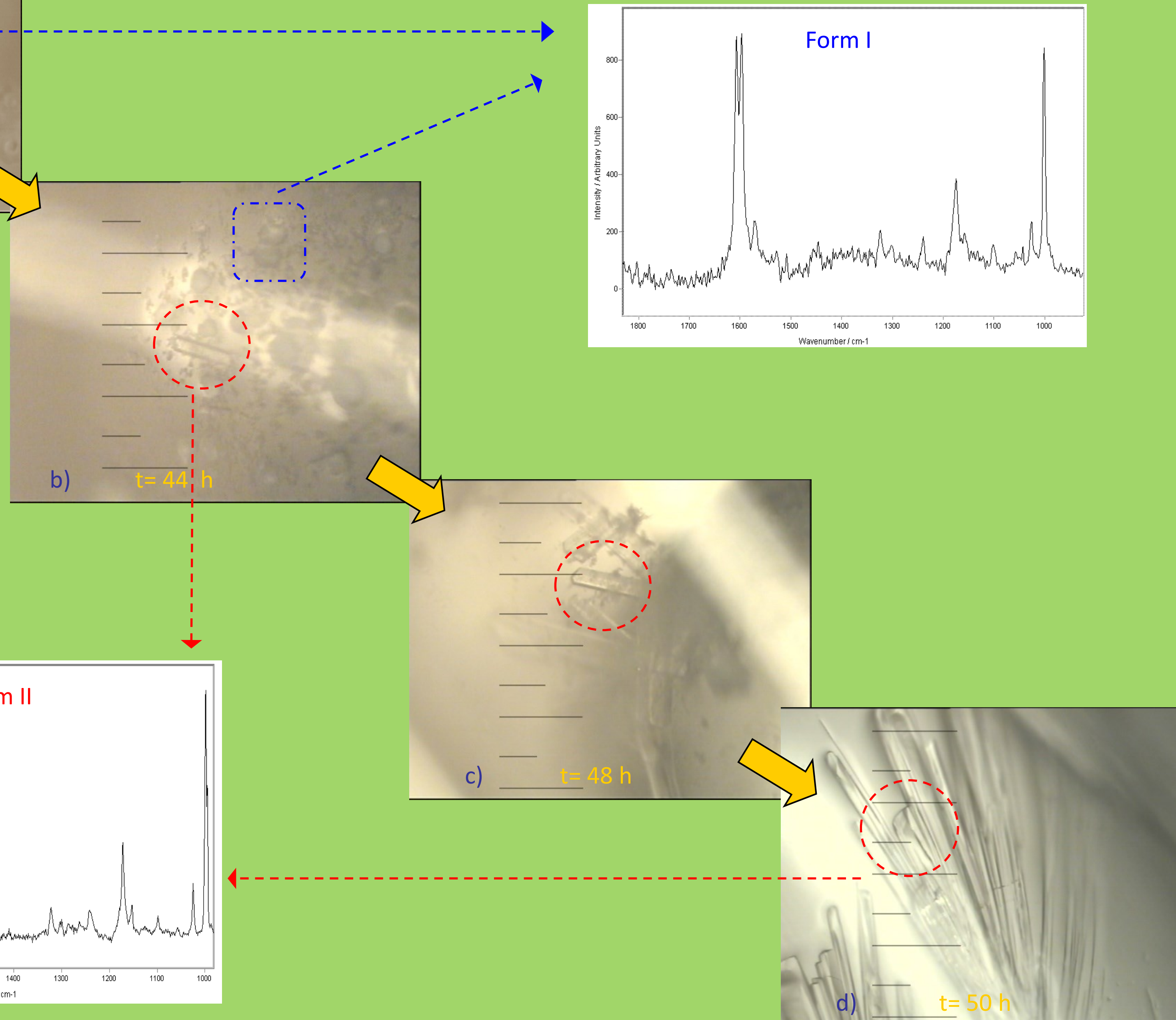
| FORM I | FORM II | FORM III | Assignments |
|---|---|---|---|
| 3057 m | 3057 m | 3057 m | substituted benzene ring |
| | | 1635 vs | mono- and para-substituted benzene rings |
| 1608 s | | 1610 w | mono- and para-substituted benzene rings |
| | 1602 vs | | |
| 1598 s | | 1597 vs | mono- and para-substituted benzene rings |
| 1444 w | 1449 w | 1452-1441 vw | $\delta$ $CH_{2,}$ $CH_3$ |
| 1228 m | 1228 m | 1225 m | para-substituted benzene rings |
| 1175 s | 1174 s | 1178 s | mono- and para-substituted benzene rings |
| | 1152 m | 1155 m | mono- and para-substituted benzene rings |
| | 1057 w | 1057 w | |
| | 1024 w | 1025 w | |
| | 998 vs | 1000 vs | mono-substitutted benzene ring |
| 923 w | 942 w | 913 vw | "ring mode" |
| | 864 m | | "ring mode" |
| 854 vw-843 w | 853 vw-843 w | 842 w | |
| 802 vw | 800 w | 807 w | Para-substituted benzene ring |
| 781 m | 785 vw | 783 w | Para-substituted benzene ring |
| 760 vw | 756 vw | 749 vw | |
| 736 w | 730 w | 729 w | |
| 680 m | 677 m | 679 m | "ring breathing" |
| 635 w | 633 w | 635 w | Para-substituted benzene ring |
| 622- 618 m | 620-618 m | 623-617 m | |
| 476 vw | 481 w | 490 vw | lattice vibrations |
| 417-405 m | 412 w | 404 m | |
| 365 vw | 366 m | 372 m-w | lattice vibrations |
| 311 vw | 313 vvw-301 vvw | 301w-288 w | lattice vibrations |
| 259 m-w | 259 m | 255 w-249 w | lattice vibrations |

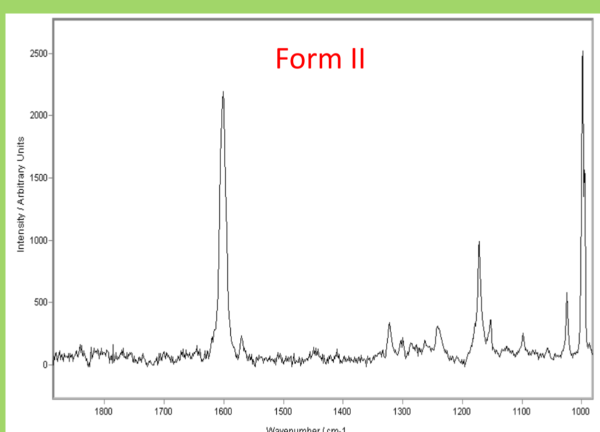
*Comparison of the Micro-Raman bands of form I, form II, and form III of Tamoxifen citrate.*

v= very;
s= strong;
m= medium;
w= weak;
sh= shoulder;
b= broad;
as= asymmetric;
s= symmetric.

# Rearrangement of form I into form II



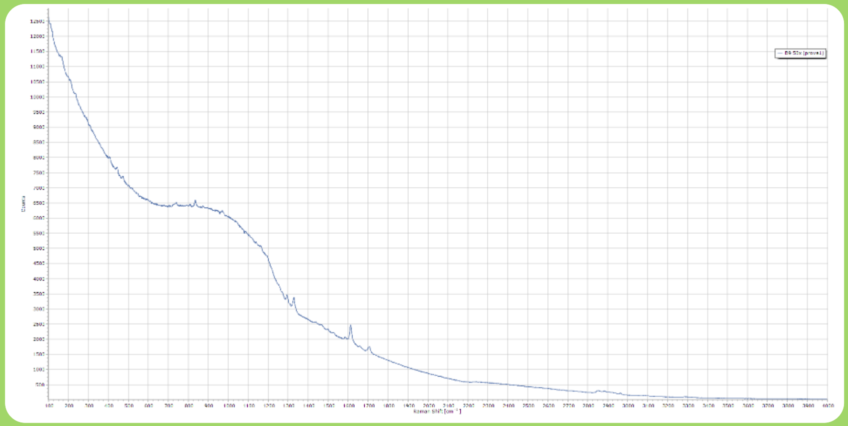



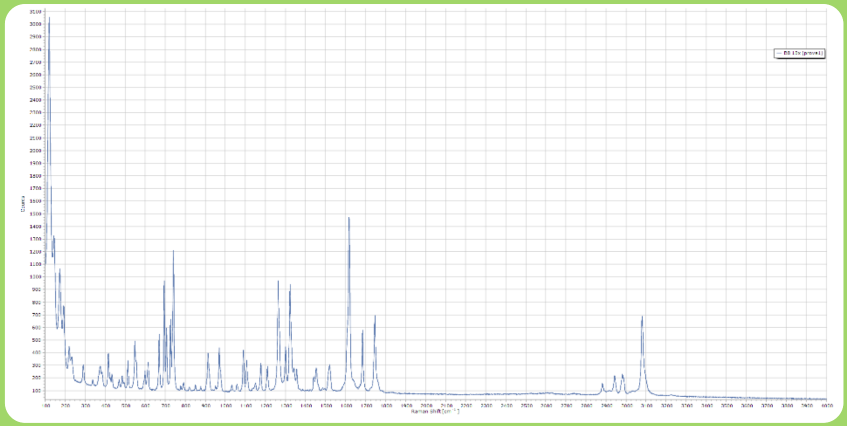
# SERS Raman Analysis

**Study of Several antimicrobials**

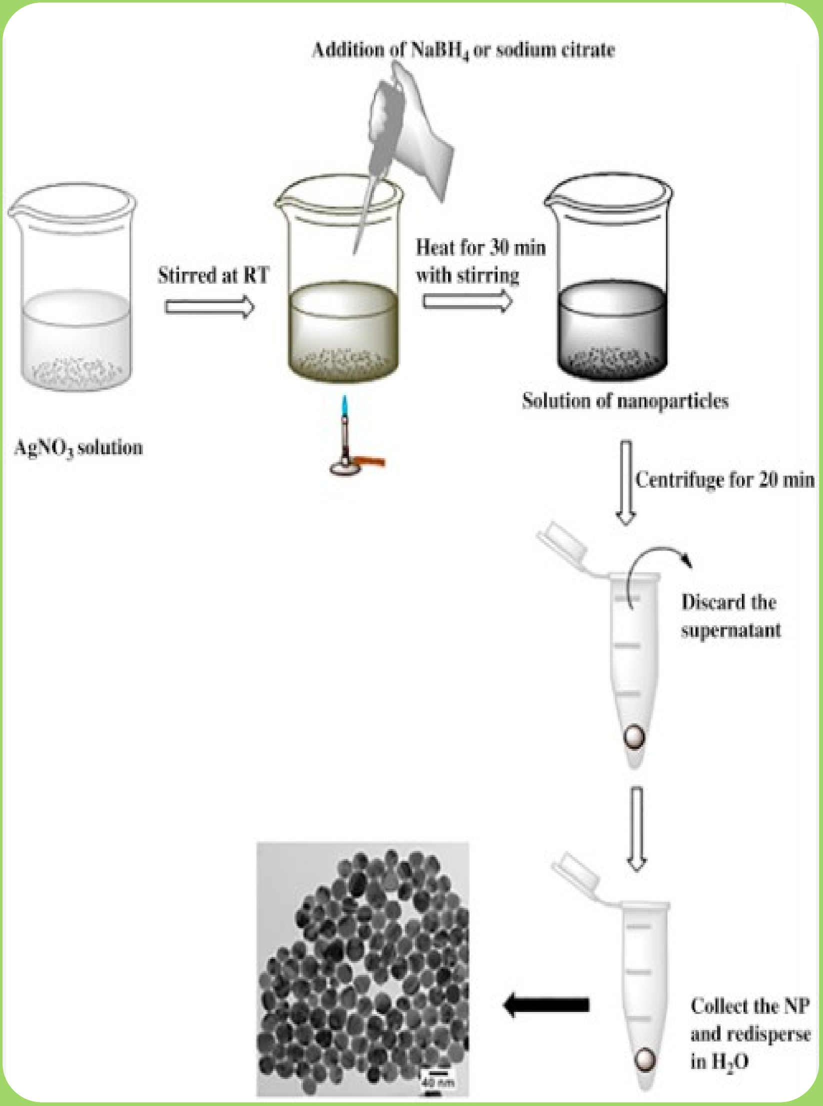
# Fluorescence in Raman Spectroscopy



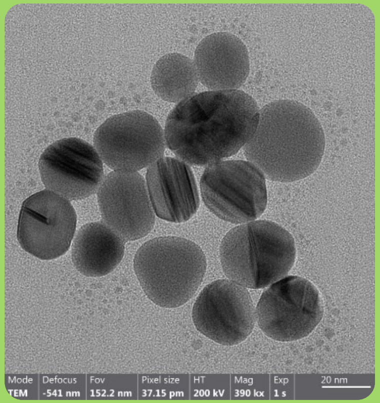
HIGH FLUORESCENCE

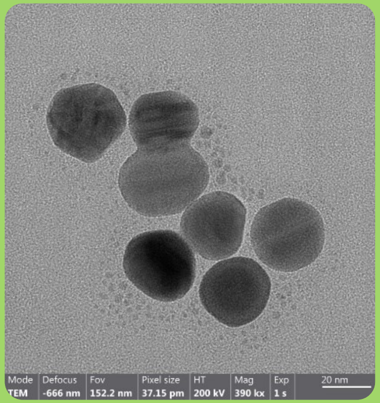


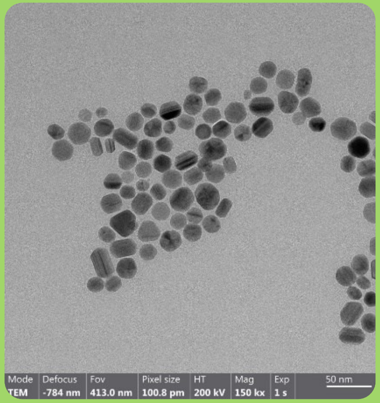
NO FLUORESCENCE



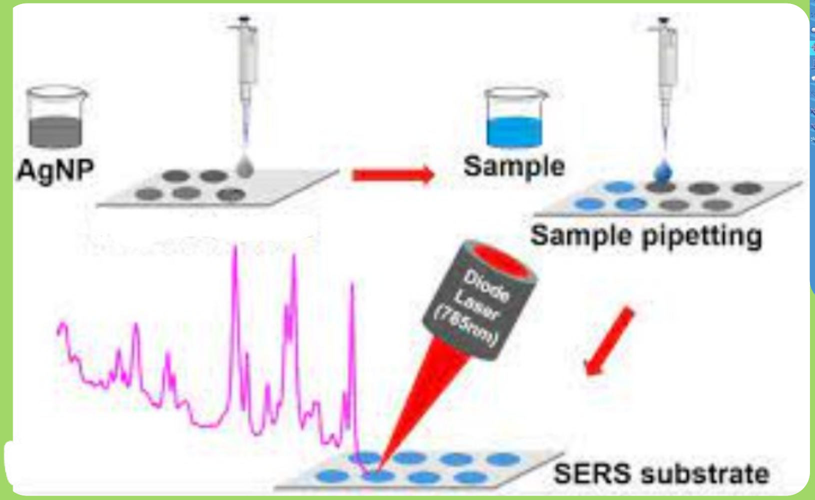

# Silver Nanoparticles – TEM images

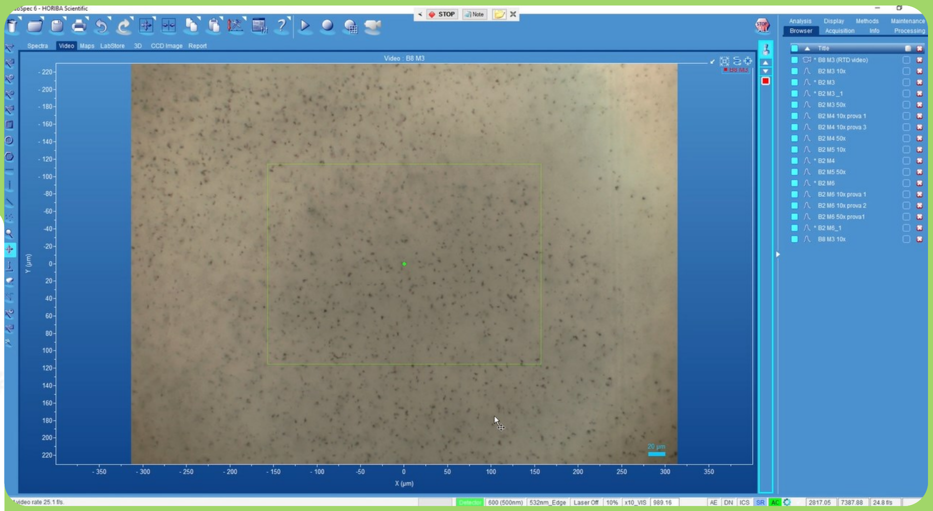


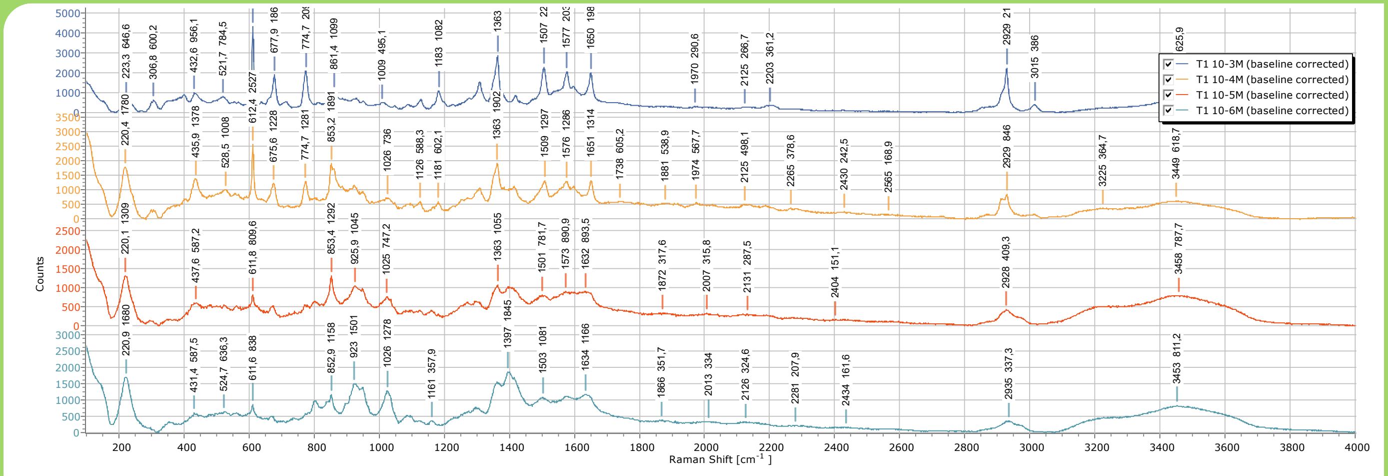



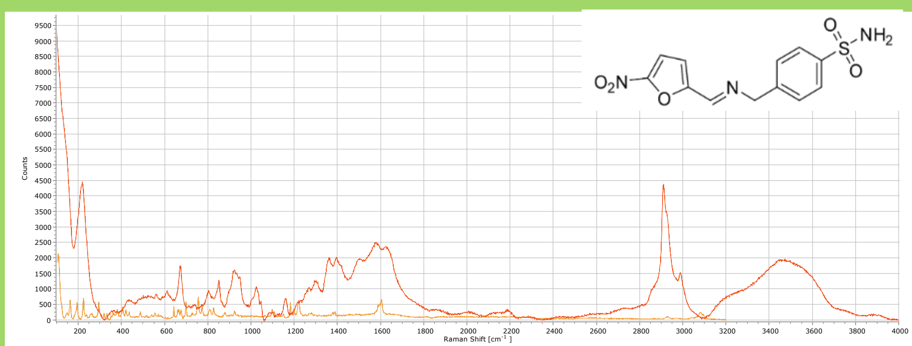
# Sample Analysis

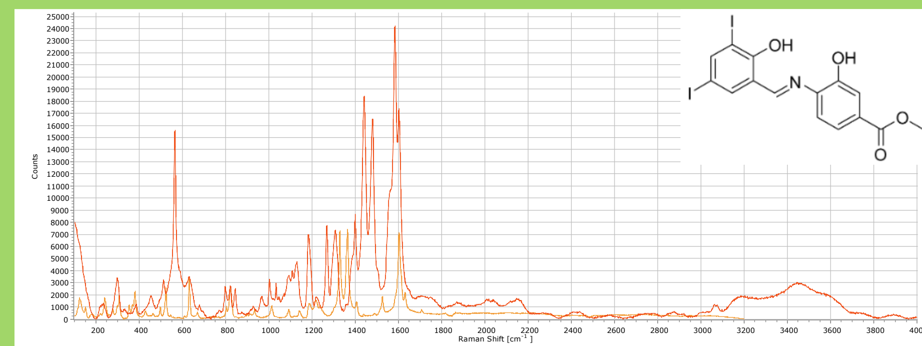


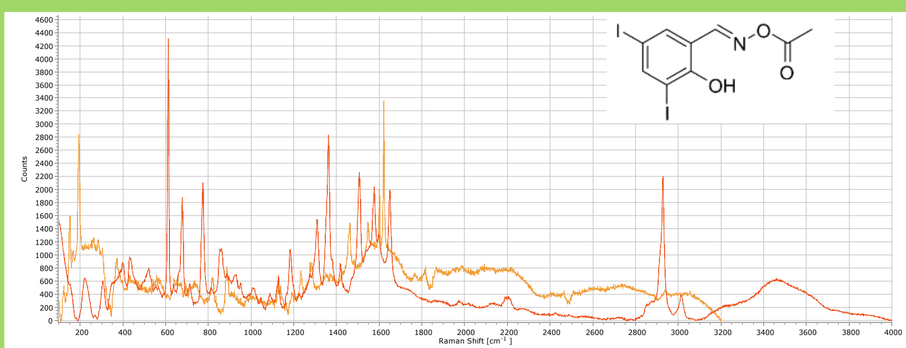



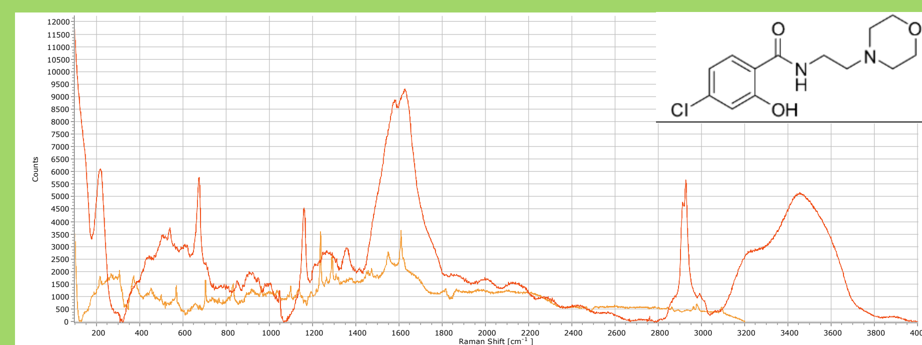


# Comparison between Raman and SERS spectra



# In situ Raman microscopy

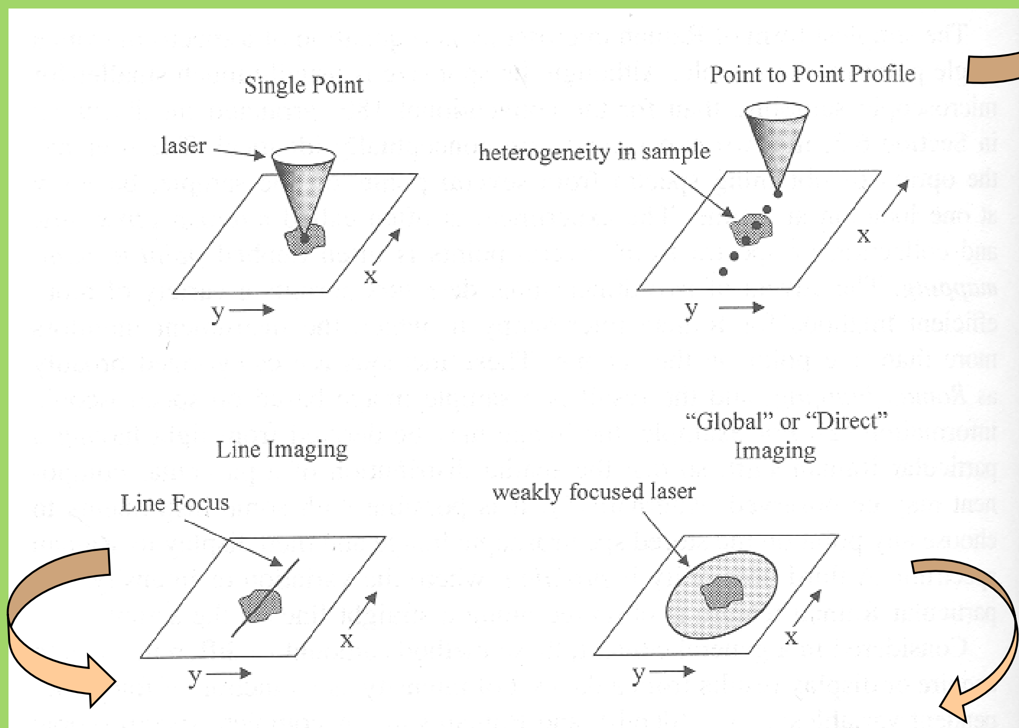
## Mapping Raman

Line Profile

Distinct point

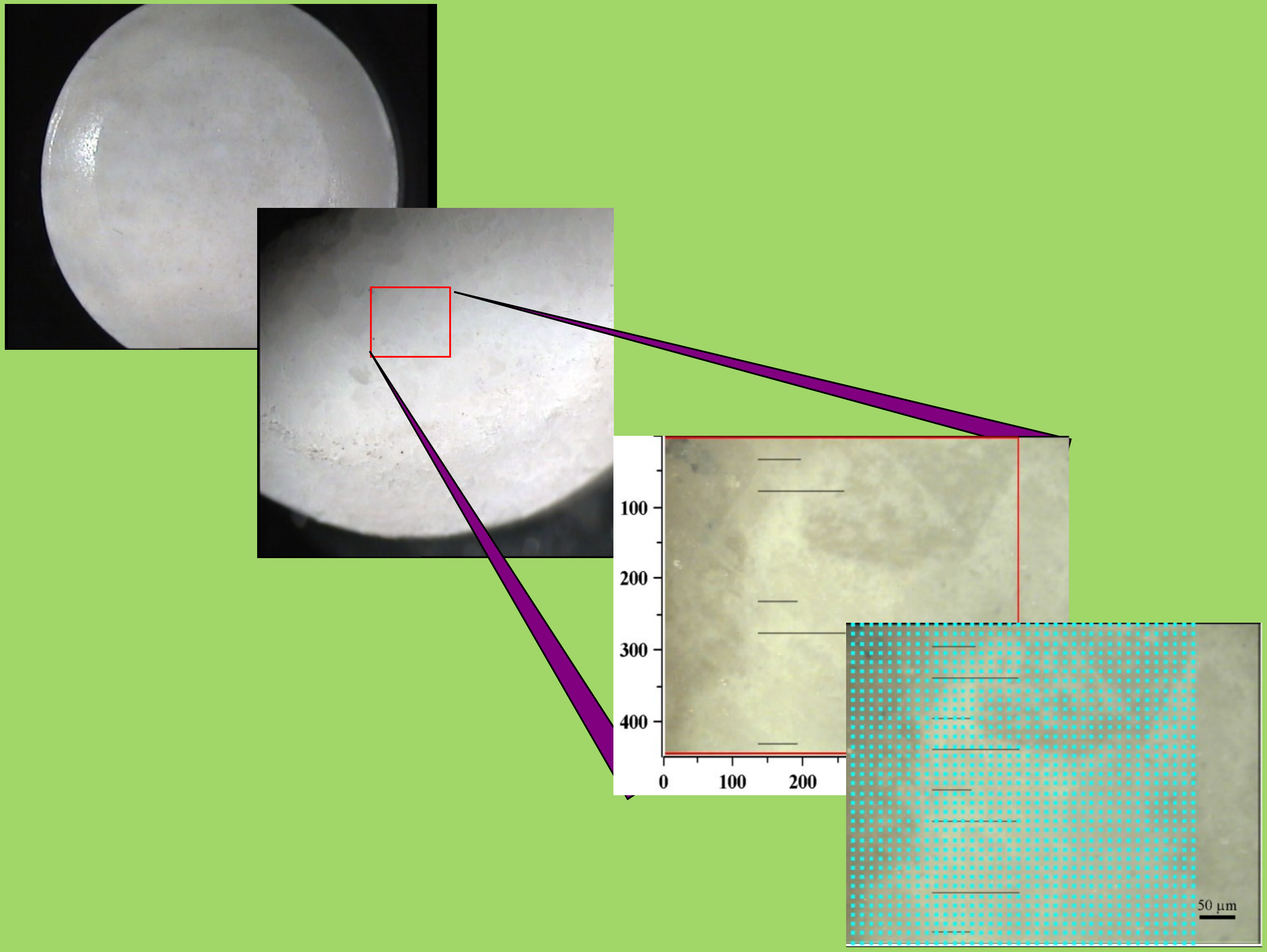
Area

Point to point profile

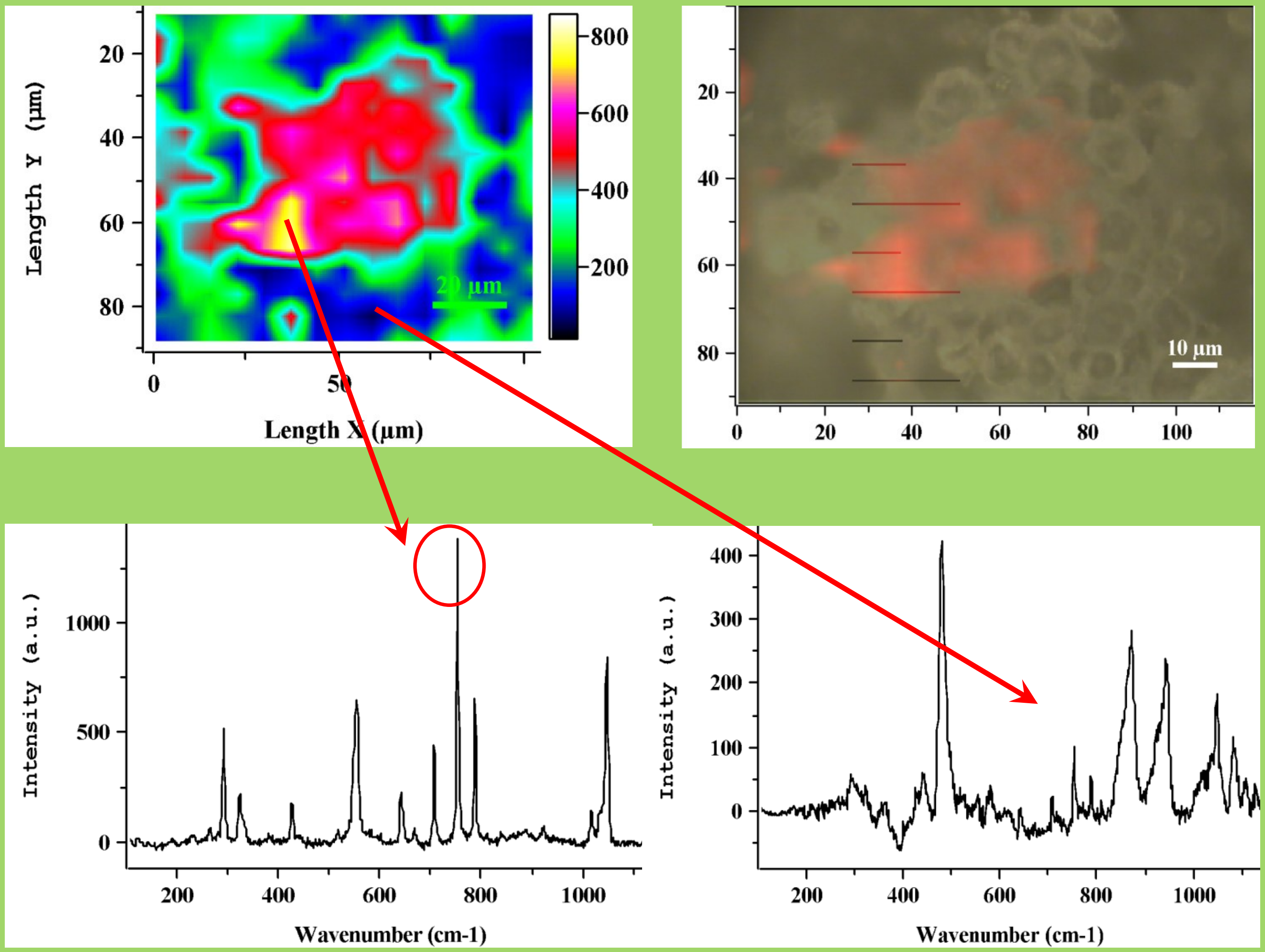



Line imaging

Direct imaging



100
200
300
400
0
100
200
50 µm



Length Y (µm)
Length X (µm)
20 µm
10 µm
Intensity (a.u.)
Wavenumber (cm-1)

# New frontier for cells characterization. Spectralomics: the Raman Spectroscopy

Maria Cristina Gamberini

Prof Graziella Pellegrini, UNIMORE
Prof Hugh J. Byrne, FOCAS INSTITUTE, TU DUBLIN

# **EU Directive-** Human Cell Atlas project

- The importance of the ability to visualise and understand processes and responses at a cellular level is accentuated by **EU Directive (2010/ 63/EU)**, which advocates the replacement, reduction and refinement (RRR) of animal experimentation, as a result of which, the development of rapid, cost effective *in vitro* techniques has been prioritised, for toxicological and pharmaceutical screening applications, amongst others.
- From 2016, **Human Cell Atlas project**: creating detailed ***maps of all human cells***, to more effective drug development, immunotherapy, and cell and organ replacement.

# *Spectralomics*

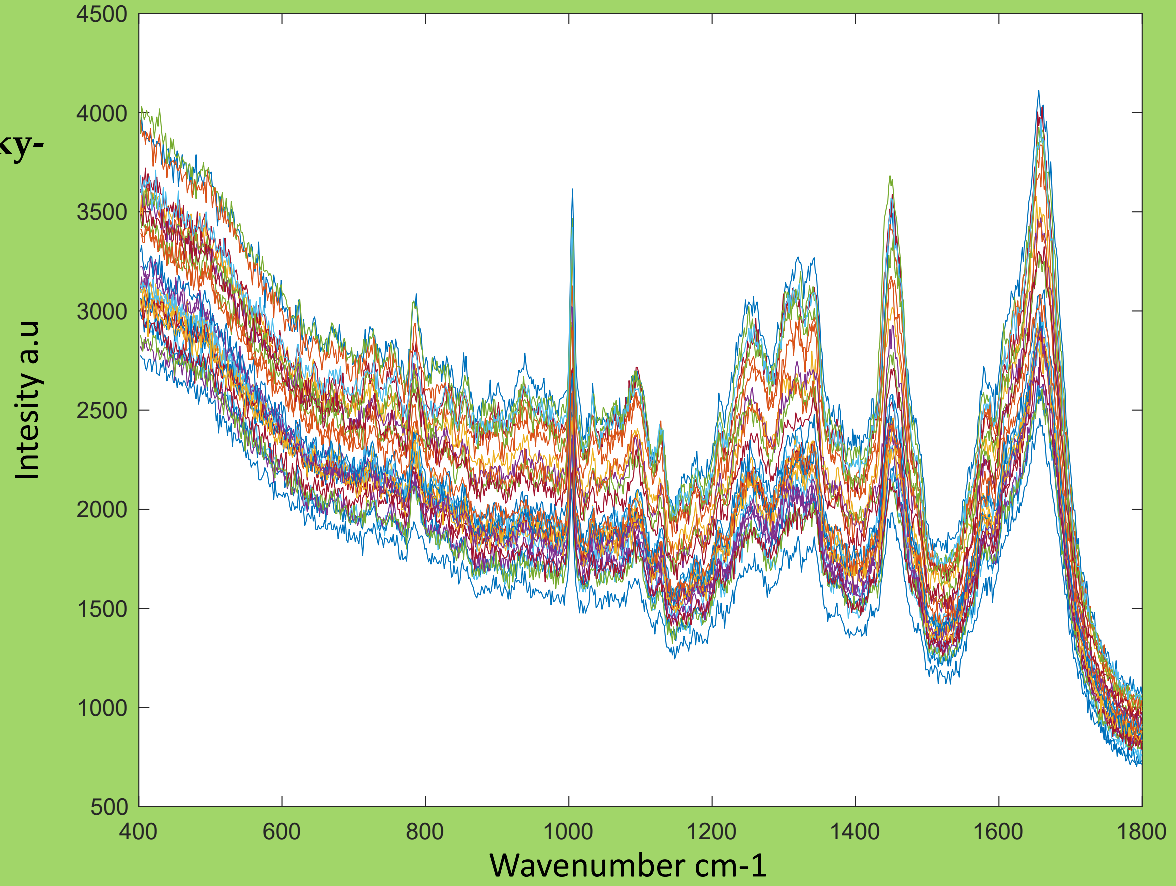
The paradigm of Spectralomics is an approach to **interpretation** and **representation of cellular events** and processes in terms of ***changes to the spectral profiles***, ***compared to controls***, or as a function of time or dose, rather than specific biochemical components or biomarkers.

In biomedical applications, it has been demonstrated that vibrational spectroscopy can **differentiate normal and diseased** (e.g. cancer, inflammation), and classify early stage diseased states, in tissue, cells, bodily fluids, and the term "spectropathology" has been coined.

# Data Analysis

1. **<u>Pre-processing</u>**

- ❖ **Filtering (polinomial Savitzky-Golay filter )**
- ❖ **Extendend Multiplicative Signal Correction (EMSC)**
- ❖ **Scaling ('Normalization')**



# CHEMICAL ASSIGNMENT

## Peaks analysis

1

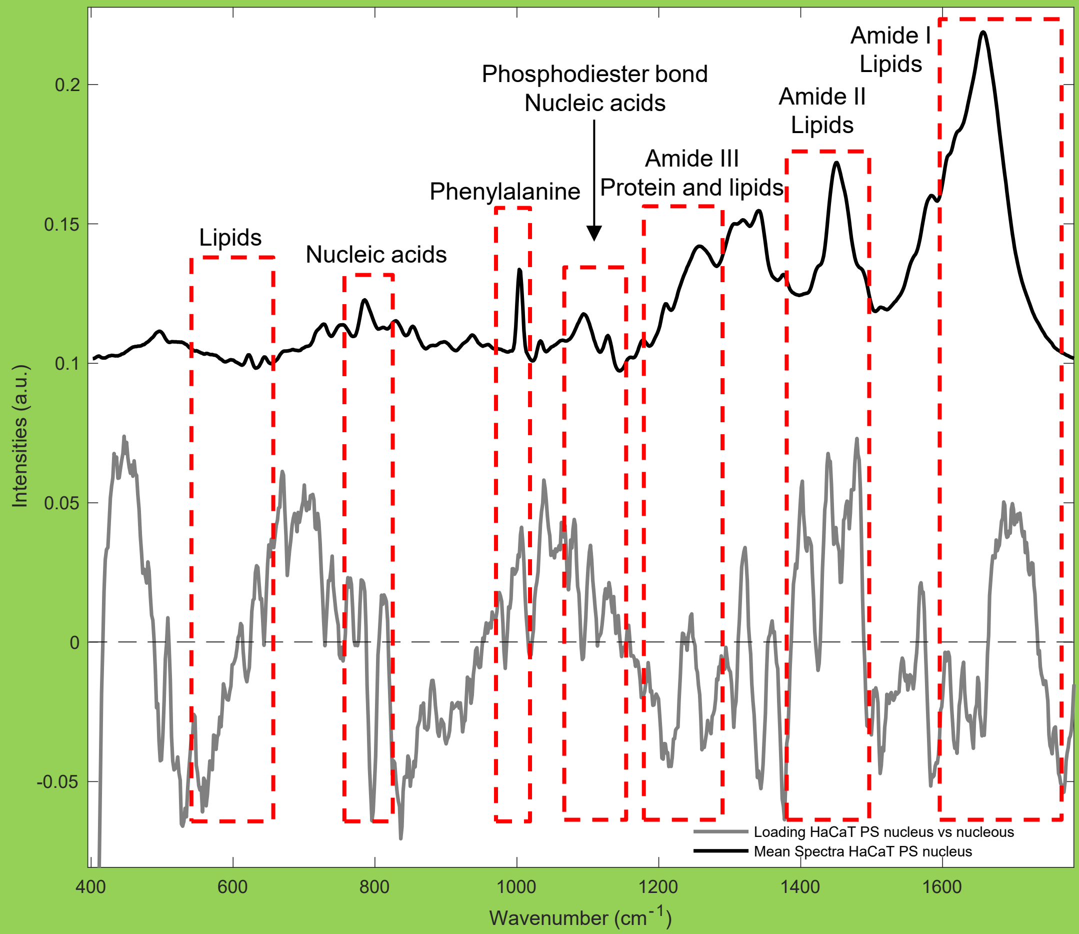
2

3

4

5

6



# POINT SPECTRA

30 cells each analizised in 3 point:

- Cytoplasm
- Nucleus
- Nucleolus

With a total of 90 spectra for each cell type took INDIVIDUALLY

90 spectra x 5 (cell types) = 450 spectra

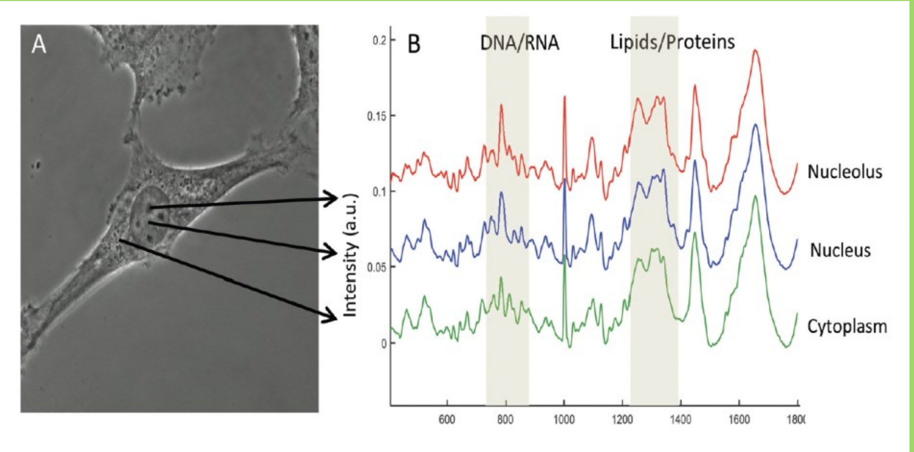
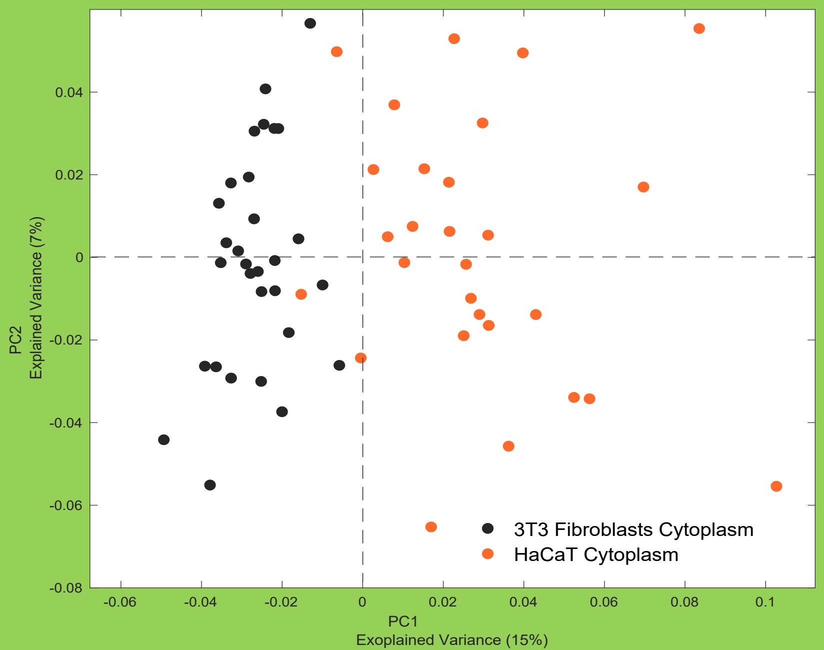


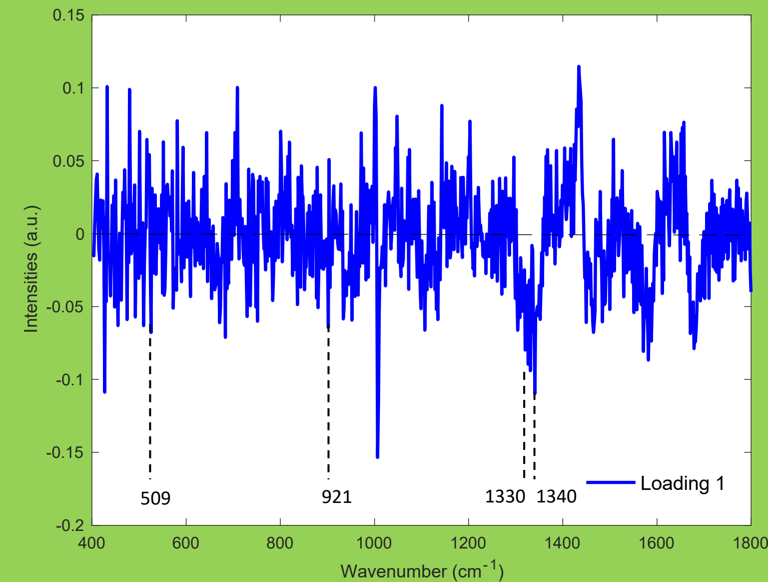

**Fig. 1** (A) A representative white-light image of MSCs. (B) Mean spectra of nucleolus (red), nucleus (blue) and cytoplasm (green) of MSCs. The cellular regions are indicated with black arrows and respective spectra are provided in (B). The spectra have been offset for clarity.

Preparation cells "FOCAS PROTOCOL" citoprecipitation on CaF2 after 24H Cells fixed with 10% formalin

# POINT SPECTRA PCA

## HaCaT and 3T3 Fibroblast - CYTOPLASM




### Loading 1

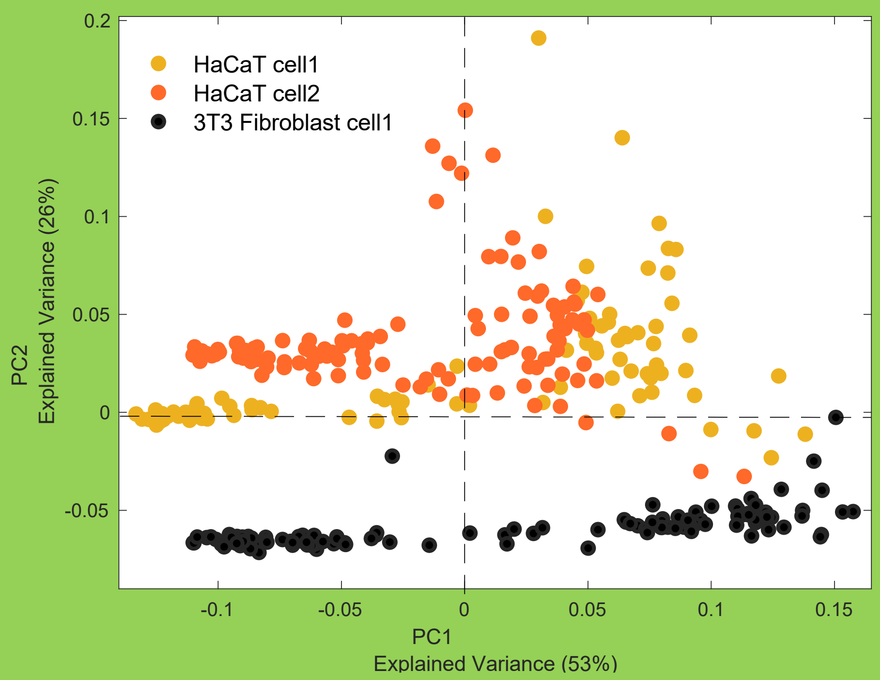
**(+) corresponding to HaCaT**
**708** Phenylalanine
**972** Lipids
**1001** Phenylalanine
**1044** Proline
**1142** Amide III
**1202** Amide III/electronic structure of nucleotides
**1438** Lipids
**1657** Amide I/ T, G, C

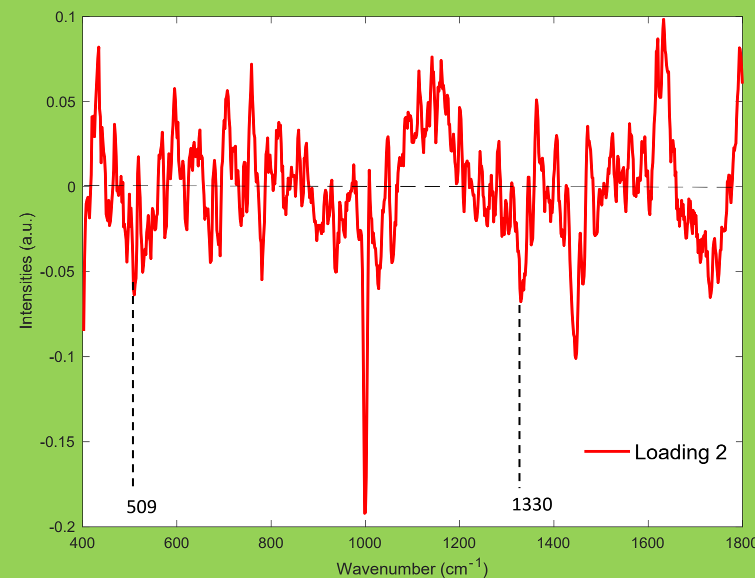
**(-) corresponding to 3T3 Fibroblast**
**509, 921, 1330, and 1340 cm$^{-1}$ Collagen**
**519** Phosphatidylserine
**1006** Phenylalanine
**1330** DNA/ phospholipids
**1581** Pehylalanine

1
2
3
4
5
6

# SINGLE CELL MAP PCA

HaCaT and 3T3 Fibroblasts




## Loading 2

**(+) corresponding to HaCaT**
**759 cm$^{-1}$** Tryptophan
**1100** fatty acid
**1160** C-C/C-N stretching (protein)
**1634** Amide I

**(-) corresponding to 3T3 Fibroblast**
**509 cm$^{-1}$** Collagen
**780** U,T,C
**1000** Phenylalanine
**1330** phospholipids and collagen
**1446** CH2 bending mode protein and lipids

# Conclusions

**Find your conclusions**

Where would you like to apply Raman Spectroscopy?

Ideas???

***The best way to start is to stop talking and start working.***

Walt Disney

***Happy to be here with you***