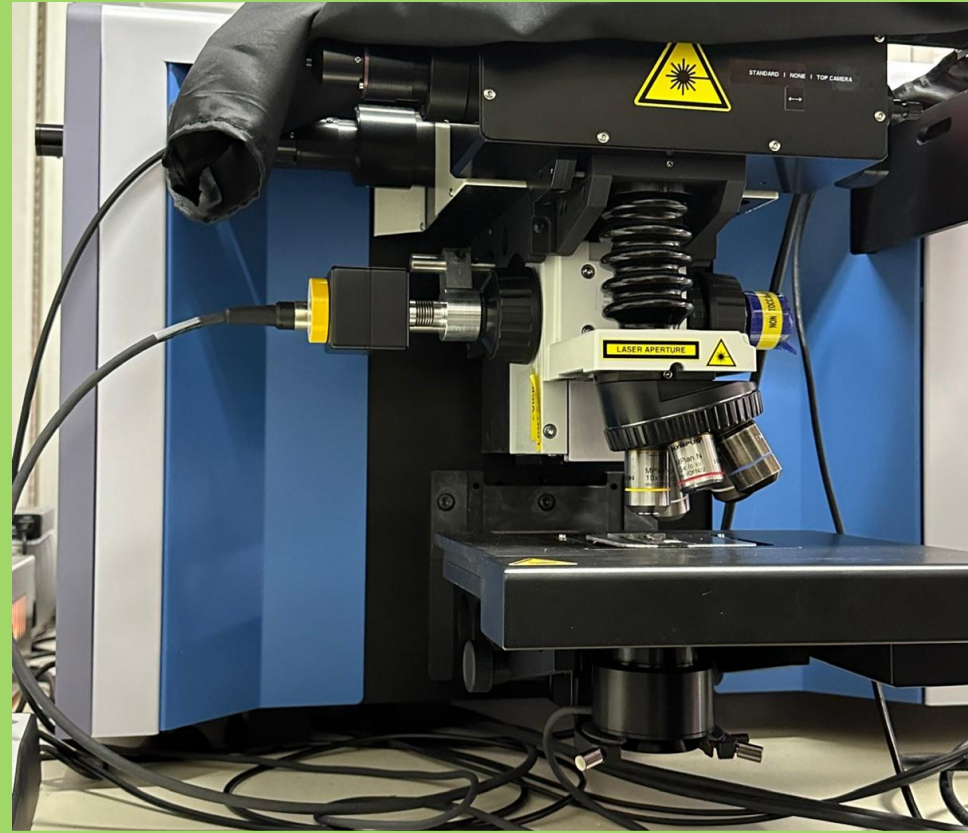


Università degli studi di Modena e Reggio Emilia
Dipartimento di Scienze della Vita

**“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:

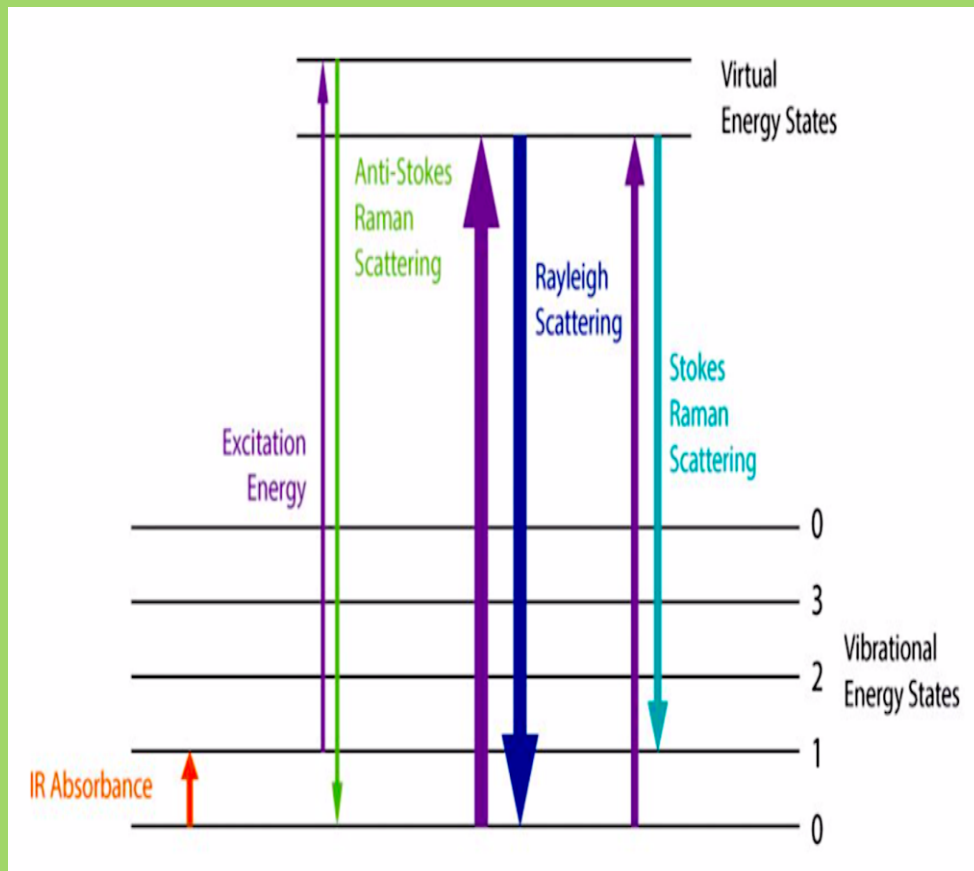
- 
- **Art and archaeology** – characterisation of pigments, ceramics and gemstones
 - **Carbon materials** – structure and purity of nanotubes, defect/disorder characterisation
 - **Chemistry** – structure, purity, and reaction monitoring
 - **Geology** – mineral identification and distribution, fluid 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

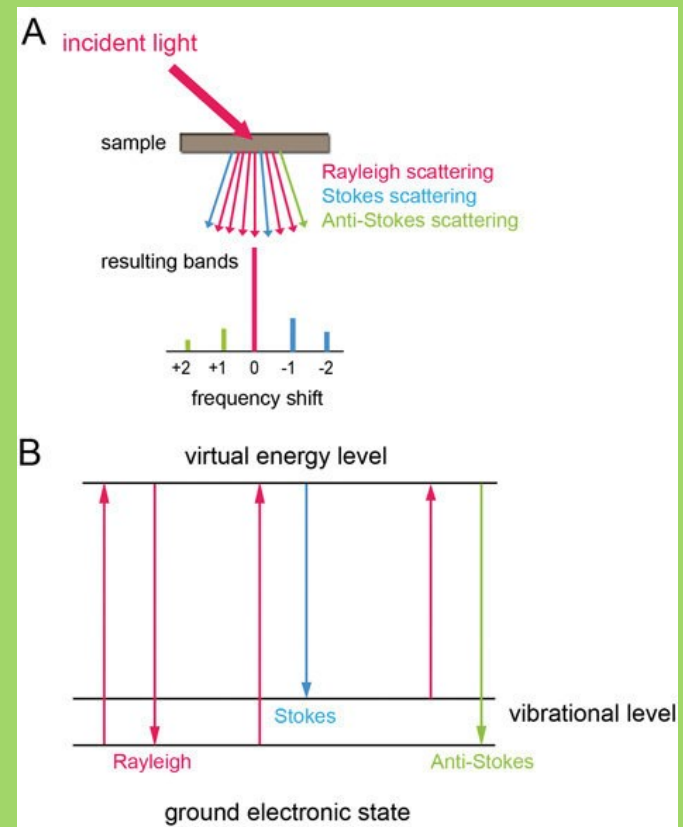
- Raman spectroscopy
- Art and archaeology
- Geology
- Pharmaceutical field
- Raman Cells

DIFFERENCES 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 possess 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).



Scattering of light: interacts without RAMAN effect 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

- **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

https://youtu.be/b_7M3Vrzo18

Spettroscopia RAMAN

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





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



As beautiful as the woman in Ancient Rome :
an overview of the Raman studies on cosmetic powders from Pompeii



UNIVERSITÀ DEGLI STUDI
DI MODENA E REGGIO EMILIA

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Historical Context

Pompeii, founded by Greek between the VII and VI century B.C., was subjected to Greek, Etruscan and Roman influences. Starting from 80 B.C. became a Roman colony (Colonia Cornelia Pompeianorum). In particular, during the imperial period, because a major place for Roman politics, the city enjoyed a favourable geographic position, near the sea and the Tiber river, improving the cultural, commercial and agricultural development.

Classical sources such as Pliny the Elder in *Naturalis Historia* and Ovid in *De Arte Amatoria* have reported ingredients and techniques for city and cosmetic Roman preparations. Locally, exotic and sophisticated ingredients are frequently quoted. Indeed, the analysis of Pompeian cosmetic powders supply a picture of a more economic and simple daily cosmetics.

Origin of the samples



Samples were taken from the remains of materials stuck to the wall of objects found during excavations. The containers subjected to this study were taken from the samples were collected by following the list:

1) private sale;

2) archaeological context;

3) independent archaeological context.

In the samples we studied, no samples were from archaeological context.

Place of Micro-Raman Spectroscopy in the analytical procedure

A complete analytical procedure was established to understand the nature of the materials and to distinguish their modification due to time. The sample is fully examined with an optical microscope (OM) to establish its homogeneity or heterogeneity and the color and size of its constituent elements. It is then observed under polarized light, which reveals the presence of minerals, organic contents or elements with characteristic fluorescence.

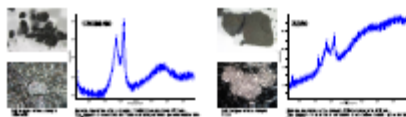
The sample is then examined with a scanning electron microscope (SEM) to determine the morphology and chemical composition of each constituent of the powder. Information is then obtained about grinding or synthesis, and about the fineness of the powder and its drying power on the skin.

The grains are characterized by X-ray diffraction (XRD) to identify and quantify the minerals present. Powder Fourier transform infrared spectroscopy (FTIR) helps also to detect the possible presence of clay minerals and particularly the presence of an organic binder in the mineral matrix. Chromatography techniques are then used for precise identification of molecules.

Finally, Raman spectroscopy is used to identify other mineral and organic products and, above all, coloring substances. We will see here the importance of Micro-Raman spectroscopy (MRS) for a complete chemical analysis of Roman cosmetic powders.

Raman and SEM/EDS measurements were performed on two confocal micro-Raman spectrometers, a notch-based Agilent Laser Raman (Laser from France, France) with the 532 nm excitation line of an Nd: YVO4 laser and an FT Spectrograph (Bior, France) equipped with a diode semiconductor laser and a backscattered light geometry. Raman modes (Agile-Vibr-Raman) were also used with 633 nm excitation line (Ar+–Kr+ laser).

Black and Dark Gray Powders : Eyebrows Enhancer

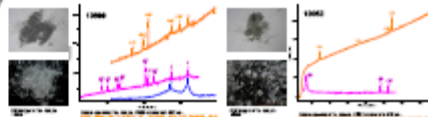


Thanks to Micro-Raman Spectroscopy, analysis showed that the content of these two objects are mostly made up of carbon-based. Particularly, the Raman technique proved to distinguish between vegetable carbon (characterized by the presence of two broad bands at about 1350 and 1570 cm⁻¹) and bone black (obtained by the pyrolysis of bone with the appearance of a band at about 970 cm⁻¹).

These black powders could be one of the black carbon-based pigments that were produced in various ways and mentioned notably by Dioscorides (*De Materia Medica*, I, 101), which, which designated as *arsenic black*, and was used to outline the eyes and eyebrows. Again, used by Pliny the Elder (*Naturalis Historia*, 33, 8), it was used to outline the eyes and eyebrows. Again, used by Pliny the Elder (*Naturalis Historia*, 33, 8), it was used to outline the eyes and eyebrows. Again, used by Pliny the Elder (*Naturalis Historia*, 33, 8), it was used to outline the eyes and eyebrows.

The most commonly used in cosmetics and available substances to color. Furthermore, the color, also related to the composition of the pigments (classified by the chemical powder) and it was also used to achieve an intense black color.

Bright Gray Powders : Eyeliner or Eyeshadow



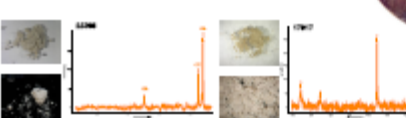
Analysis of samples of the powder contained in these two vessels showed it to be a mixture of calcium, carbonate and silica in one case and a mixture of argillaceous, carbonate, gypsum and calcium in the other case.

The gray could come either from a small quantity of gypsum or from the presence of calcium-based particles. In all cases, Micro-Raman Spectroscopy helps us to characterize these small particles in the white powder, major component of the cosmetic, by particularly focusing on the peaks.

Indeed, these calcium-based particles are the use of a black preparation called *arsenic black* or *arsenic black*. It was used to outline the eyes and eyebrows. Again, used by Pliny the Elder (*Naturalis Historia*, 33, 8), it was used to outline the eyes and eyebrows. Again, used by Pliny the Elder (*Naturalis Historia*, 33, 8), it was used to outline the eyes and eyebrows.

The most commonly used in cosmetics and available substances to color. Furthermore, the color, also related to the composition of the pigments (classified by the chemical powder) and it was also used to achieve an intense black color.

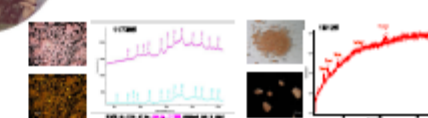
White Powders : Foundation Cream



The material inside these two containers are very fine white powder. In the first case it is a finely ground calcium and in the second case it is a mixture of carbonate and argillaceous. Calcium is described as being "used by women to whiten their skin" (Pliny the Elder, *Naturalis Historia*, 33, 8) and as a white powder (Pliny the Elder, *Naturalis Historia*, 33, 8).

The presence of lead carbonate is rather new in analyzed samples than the Roman period, but it offers new evidence in the analysis. Pliny the Elder described in detail not only the properties of this pigment, but also its advantages and its use in both of them, emphasizing that calcium has the same properties as the other white pigments that could be used as makeup. "Lead is the subject of art, in addition, women use it to whiten their complexion. Taken internally it is a poison" (Pliny the Elder, *Naturalis Historia*, 33, 8). Moreover, presence of calcium could be useful for clearly identify a cosmetic product, since calcium was never used in red pigments.

Pink Powders : Blush Cheek Color



These two examples show that pink makeup could be a mixture of several products. In one hand it is hematite (or ochre) pigment with a white pigment, probably calcium, and in the other hand it is a "magnesian pigment", a synthetic substance with a high concentration of aluminum compounds, here a clay, that serves as a base for the color-bearing molecules.

For these samples, MRS shows a promising technique to characterize the color of the pink color. Firstly, the presence of the very small quantity of hematite in the mixture, of course enough to give a pink color to the cosmetic, but not sufficient to be characterized with clarity by XRD, is given by Micro-Raman Spectroscopy (MRS) thanks to the possibility of focusing on red pigments. Secondly, the use of carbonaceous materials (pyrolytic carbon) is observed. In addition, the Raman signal of adsorbed molecules (pyrolytic carbon) and indicate the use of plant of the Roman period, such as resins, to color the skin. Typically the cosmetic pink color was colored from the expensive purple, it was the famous porphyria. Indeed, the purple pigment, obtained from the presence of an ammonium salt, Pliny the Elder, (*Naturalis Historia*, 33, 8), his species of "purple of Miletus" (purple) with the Arabic dictionnaire (L. Baudouin), such dye could have been employed as economic alternative, it can be thought that one with one of the frequent inorganic colors.

Conclusions

The chemical analyses of the cosmetic powders from Pompeii give a good idea of the diversity of materials and complexity of the cosmetic preparations at Roman age. These few examples are representative of a larger number of objects.

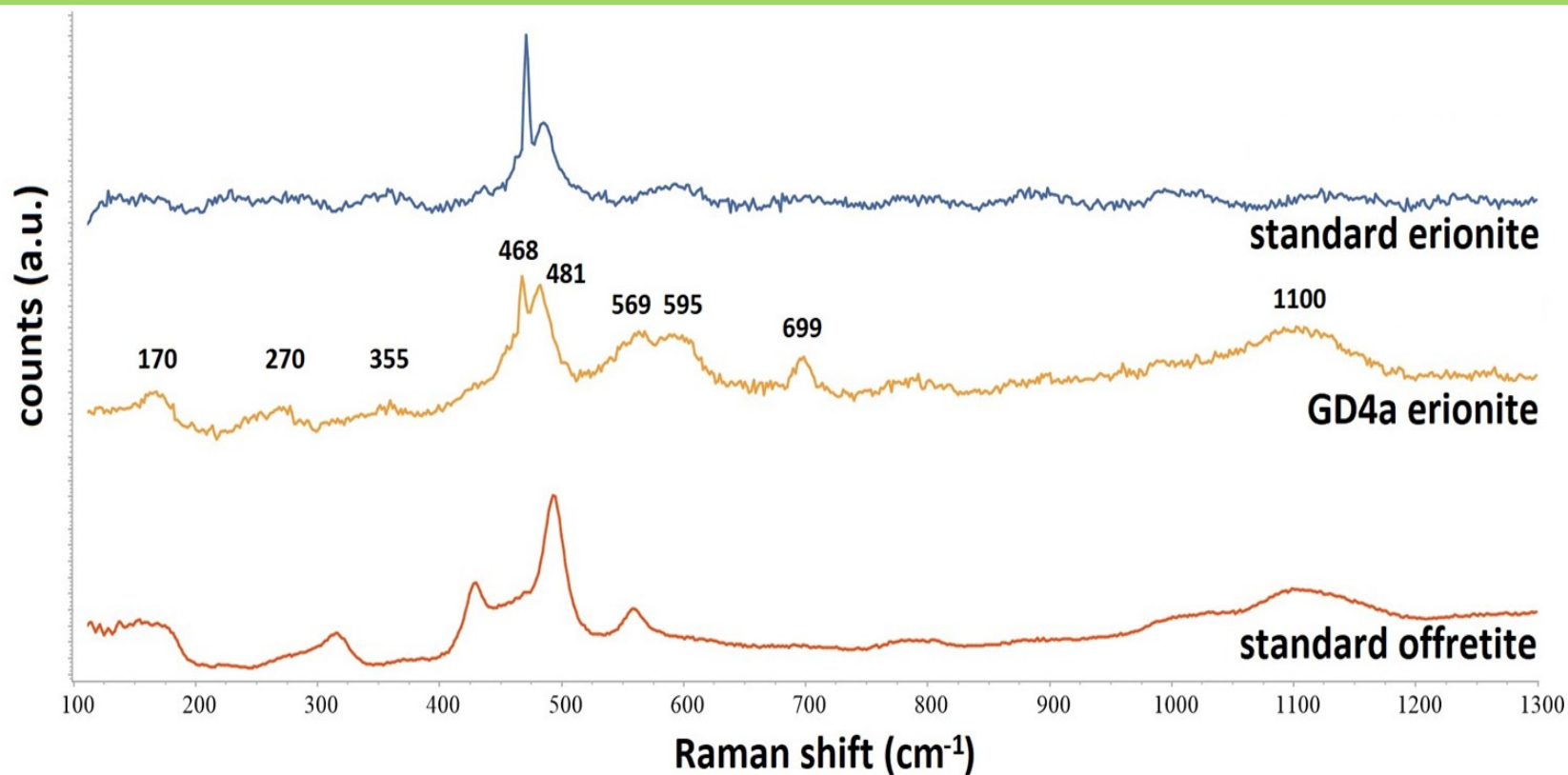
The analytical results show the important place of Micro-Raman Spectroscopy in the analytical protocol. This technique offers advantages given by none else. Also the possibility of identification of mineral present in the quantity with a very small size of particles. With SEM/EDS technique, it is also possible to detect and describe specific elements.

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- E. Van Elslande, S. Luciani, A.S. Le Po, Journal of Raman Spectroscopy 1301-1306 (2008) 29.



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)



2024/1441

21.5.2024

DECISIONE DELEGATA (UE) 2024/1441 DELLA COMMISSIONE

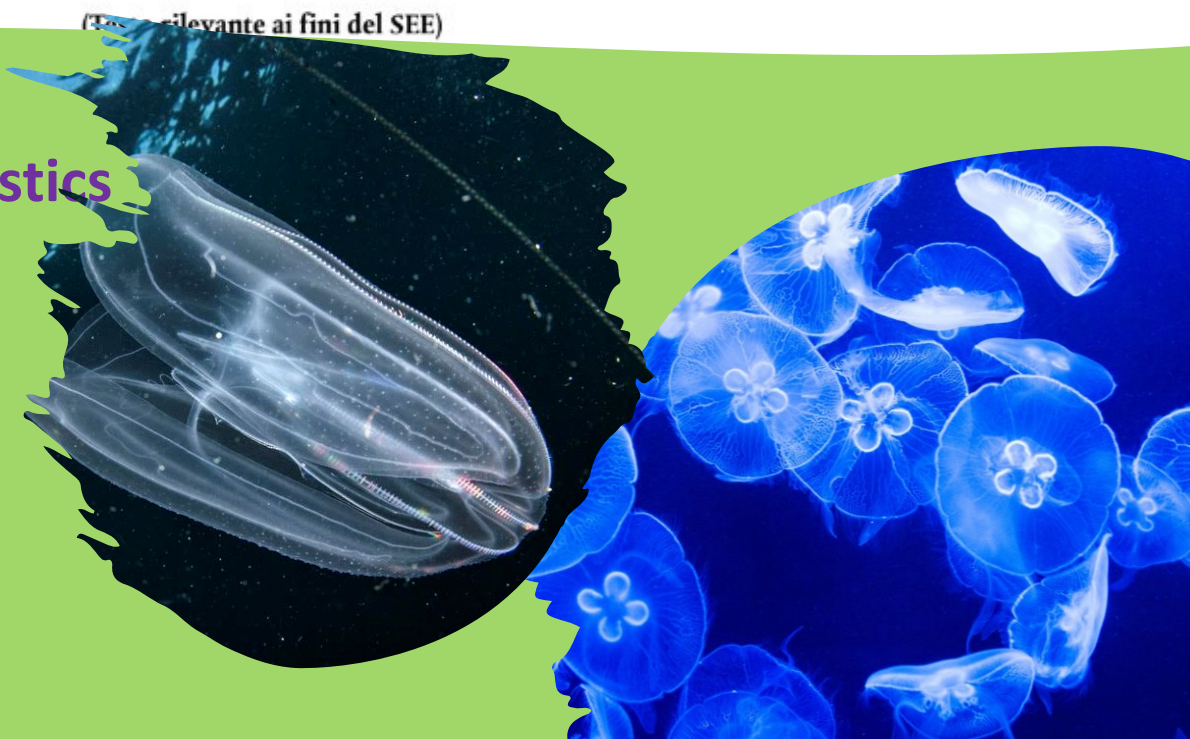
dell'11 marzo 2024

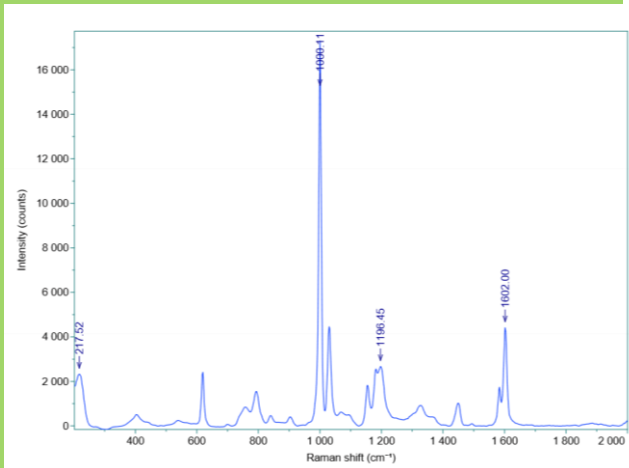
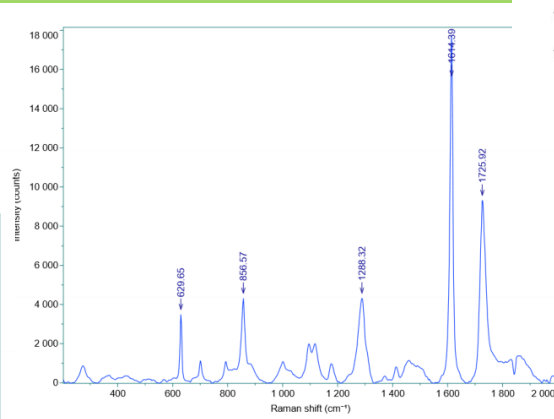
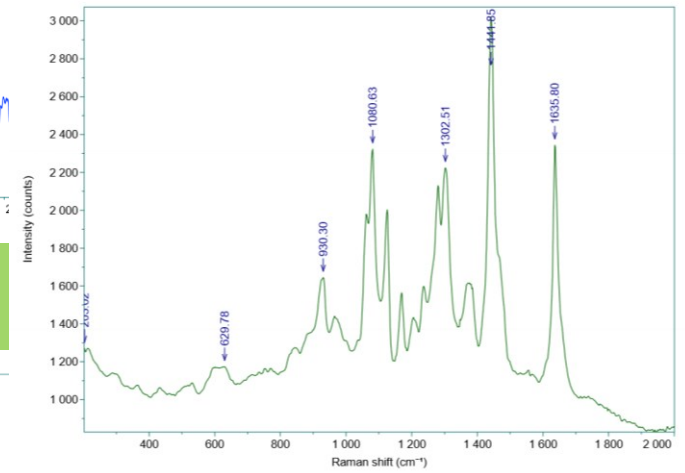
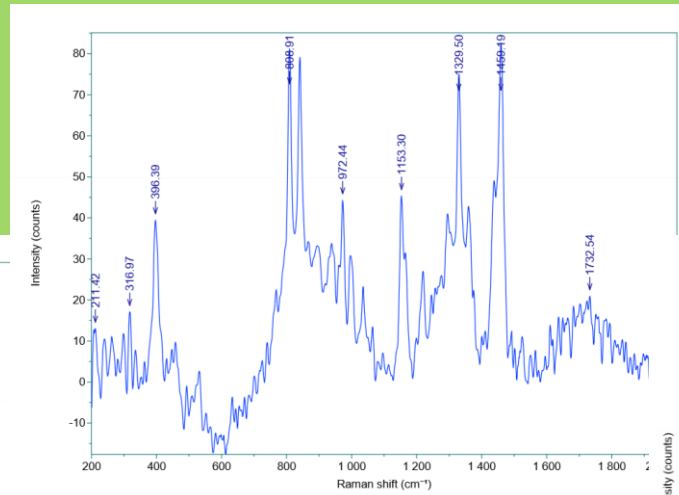
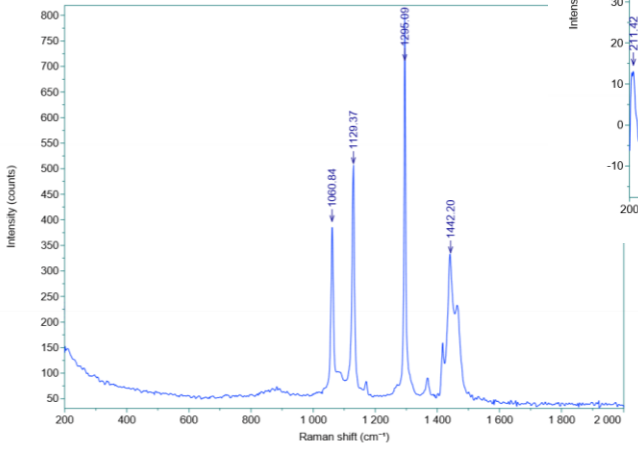
che integra la direttiva (UE) 2020/2184 del Parlamento europeo e del Consiglio stabilendo una metodologia per misurare le microplastiche nelle acque destinate al consumo umano

[notificata con il numero C(2024)1459]

(Testo rilevante ai fini del SEE)

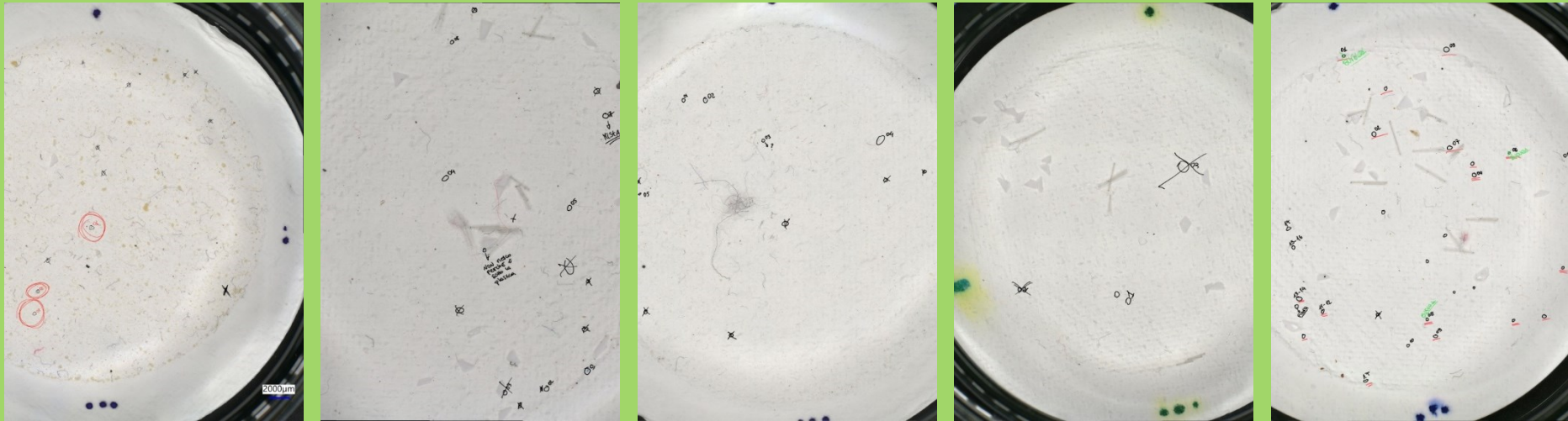
- **Sea degraded plastics**
- **Jellyfish**
- **Ctenophora**
- **Wormdogs**





Microplastics Raman spectra:

PA, HDPE, PET, PP, PS

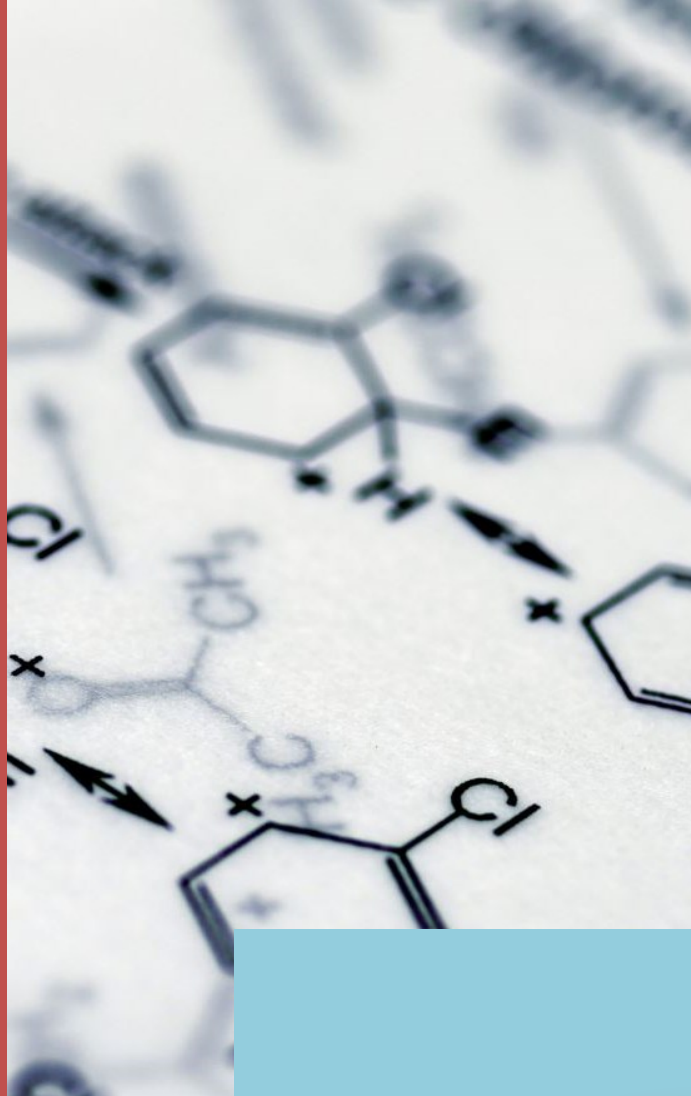


From the optical images, 80 microplastics were found
36 microplastics were Raman analyzed

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

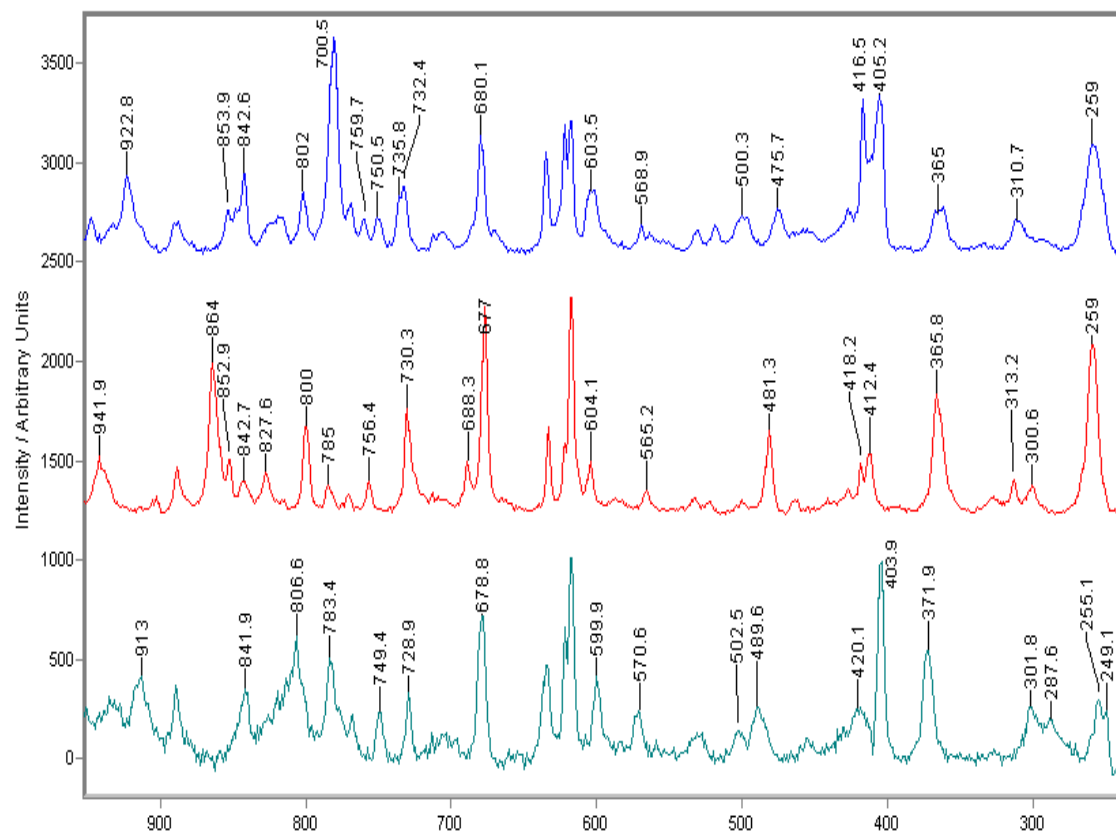
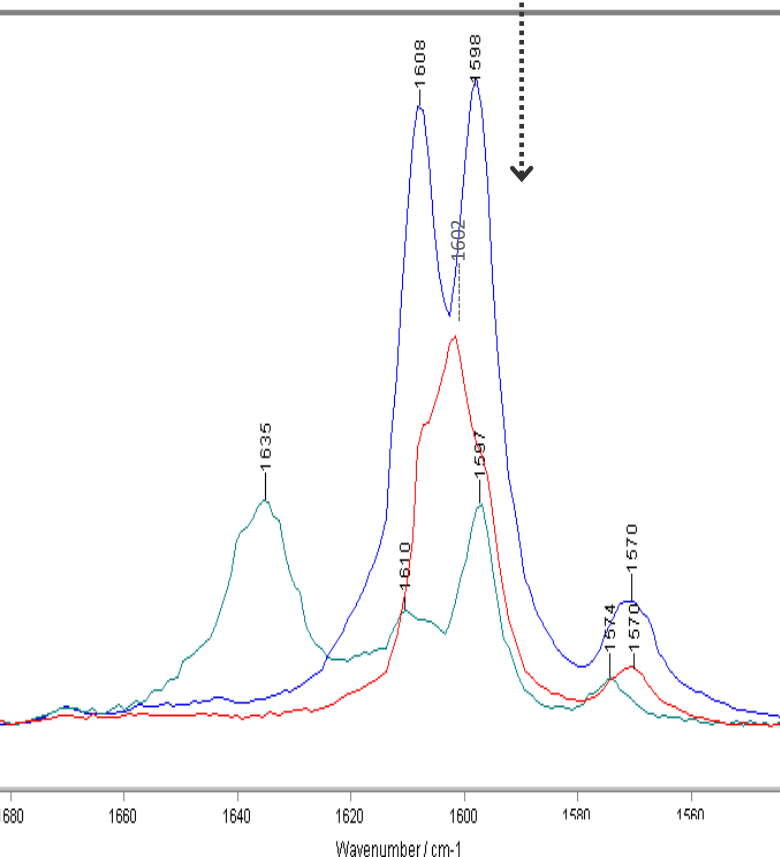
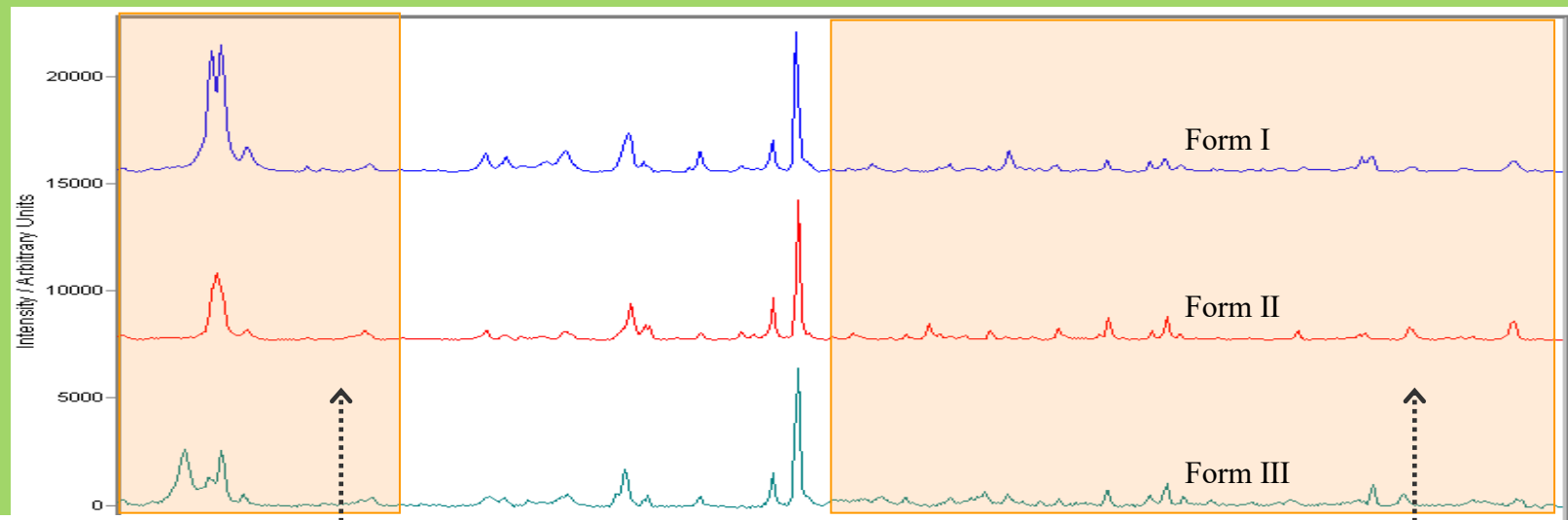
Active Pharmaceutical Ingredients

- 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**

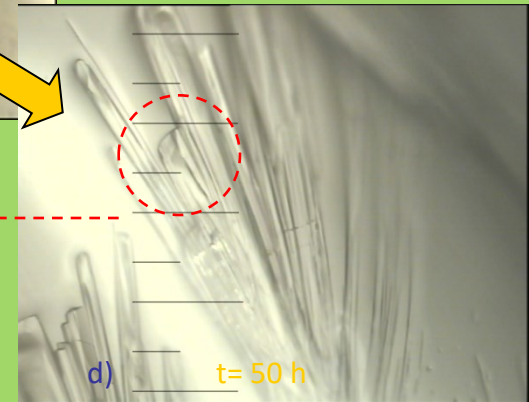
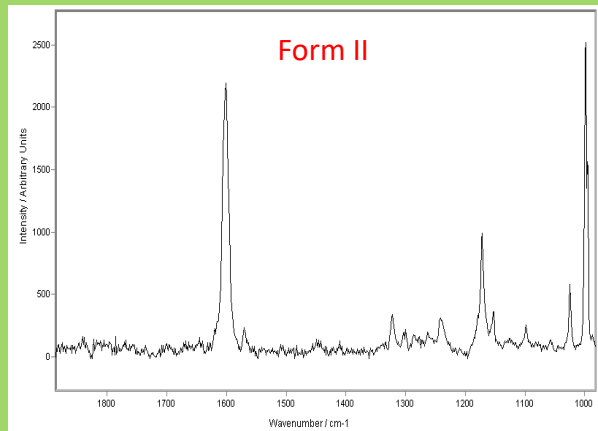
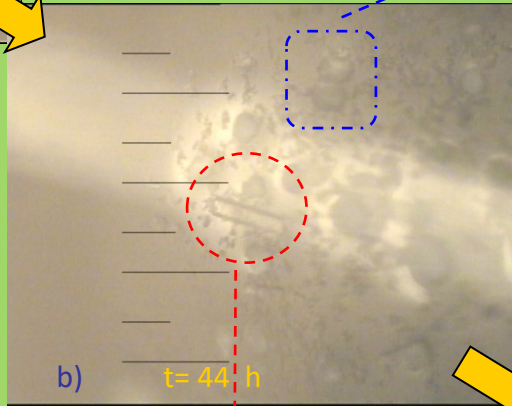
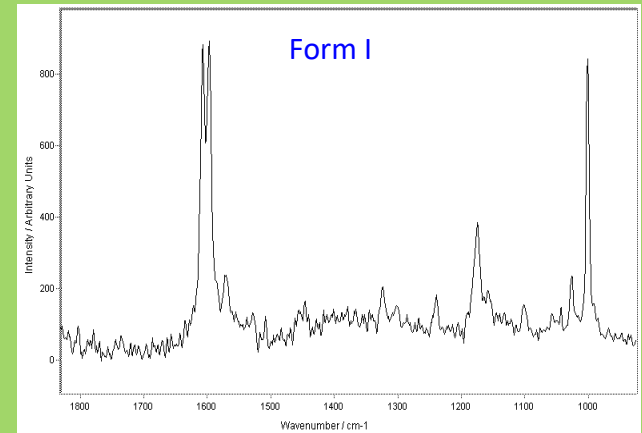
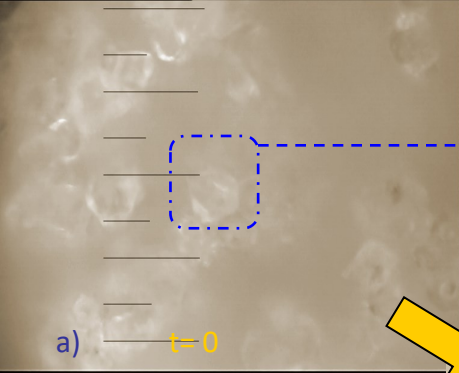


*Comparison of
the Micro-Raman
bands of
form I, form II,
and form III
of Tamoxifen
citrate.*

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	δ 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-substituted 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

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

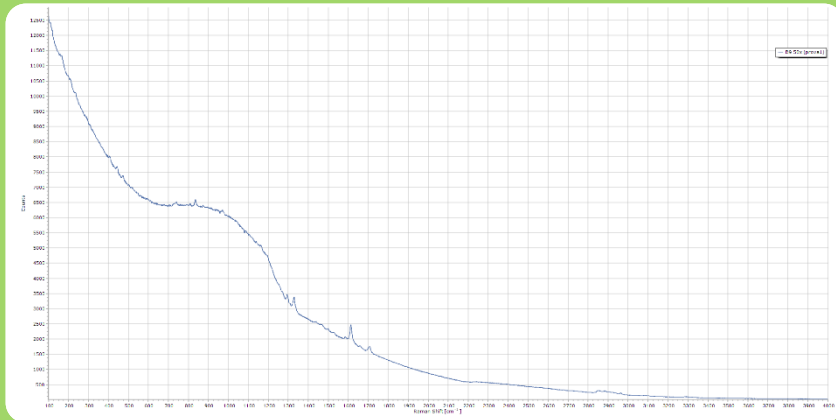
Rearrangement of form I into form II



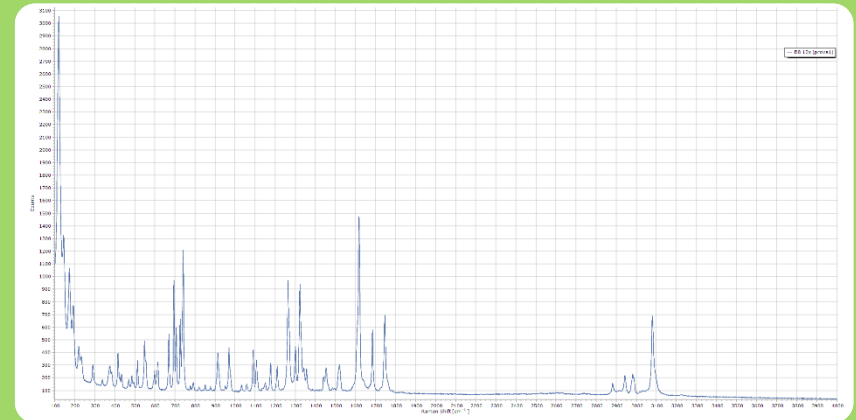
SERS Raman Analysis

Study of Several antimicrobials

Fluorescence in Raman Spectroscopy

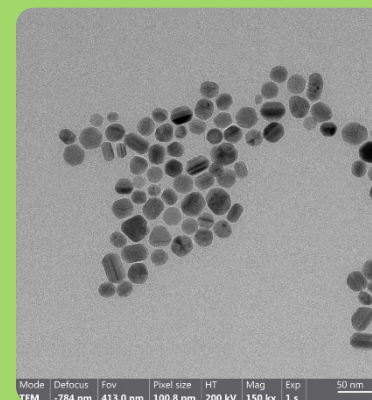
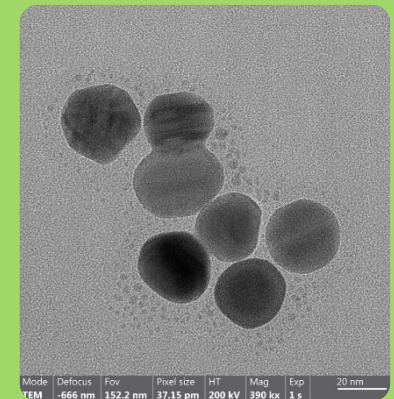
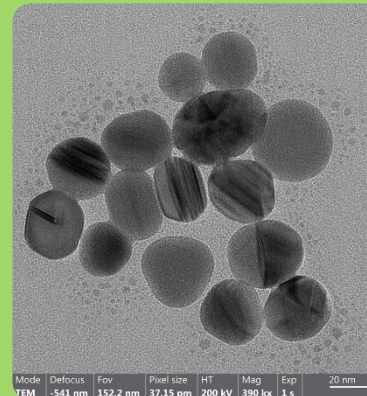
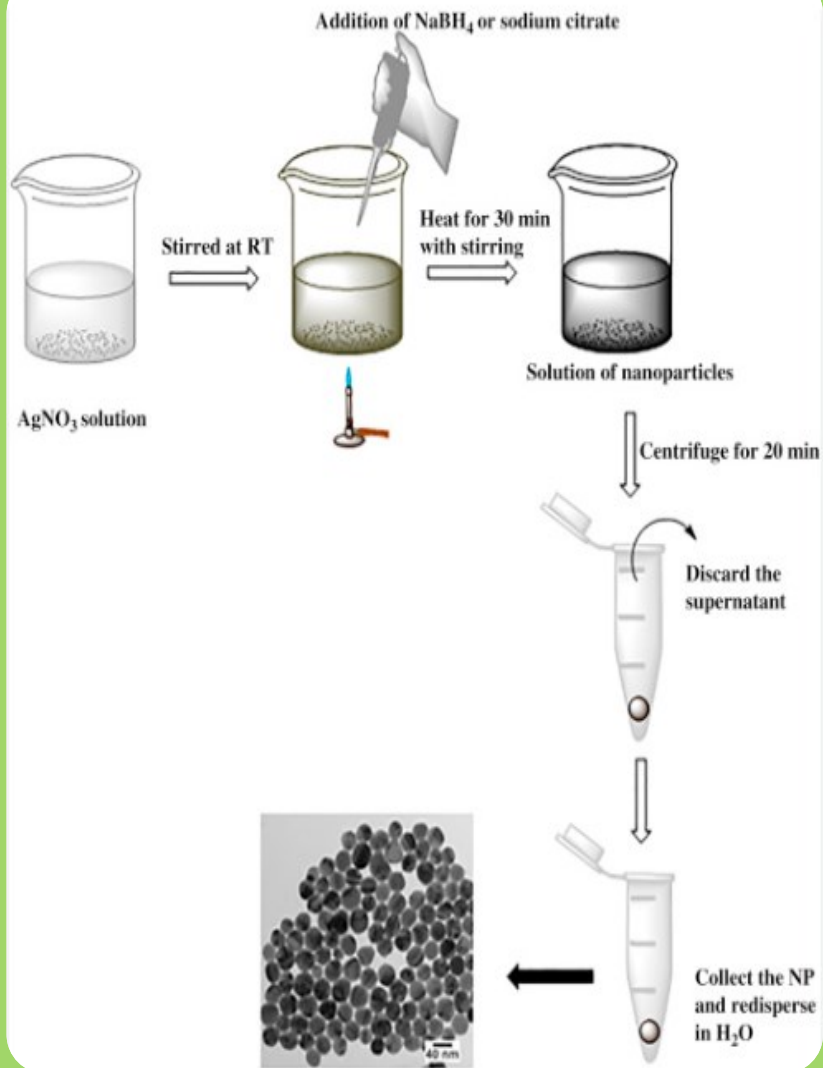


HIGH FLUORESCENCE

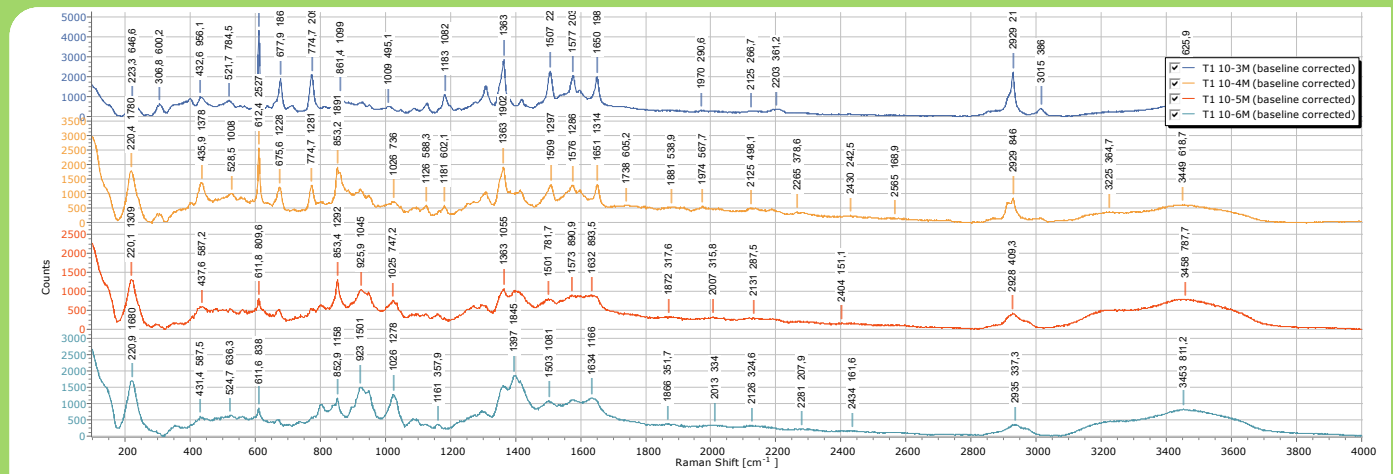
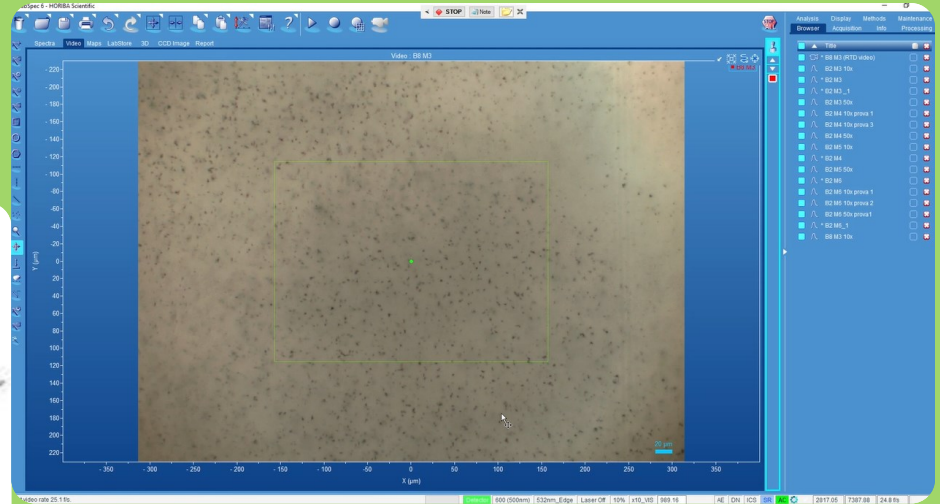
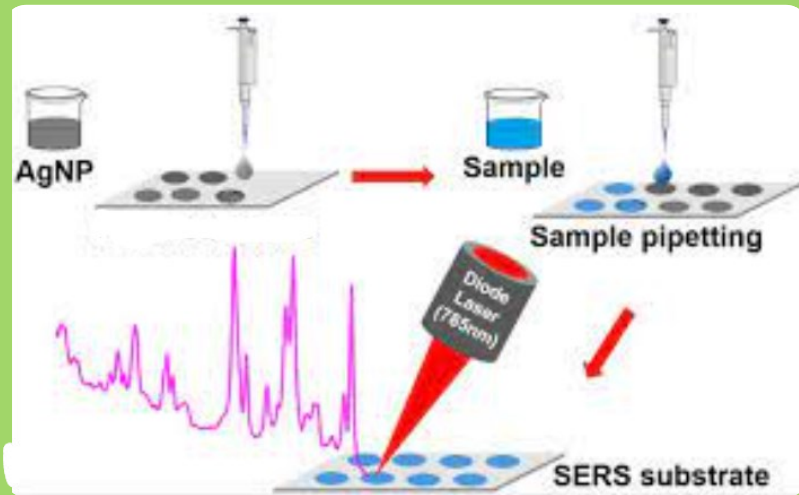


NO FLUORESCENCE

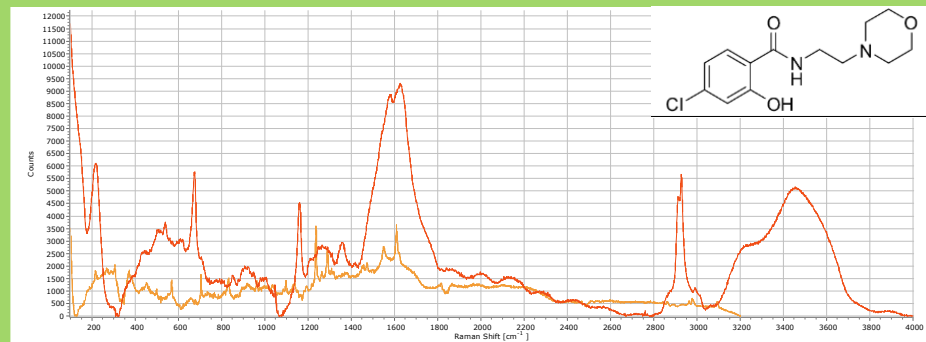
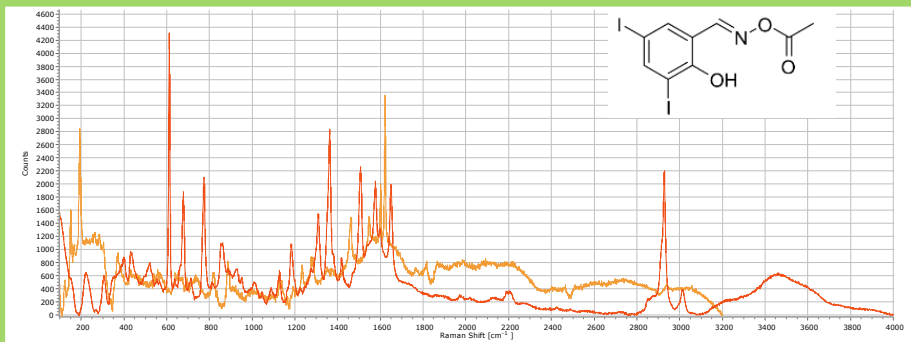
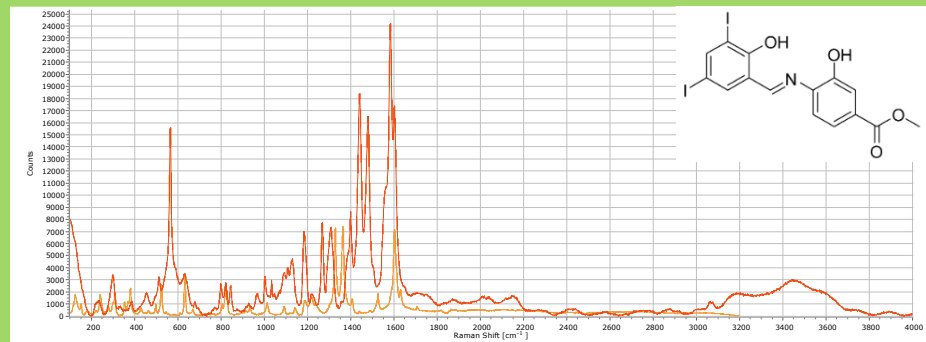
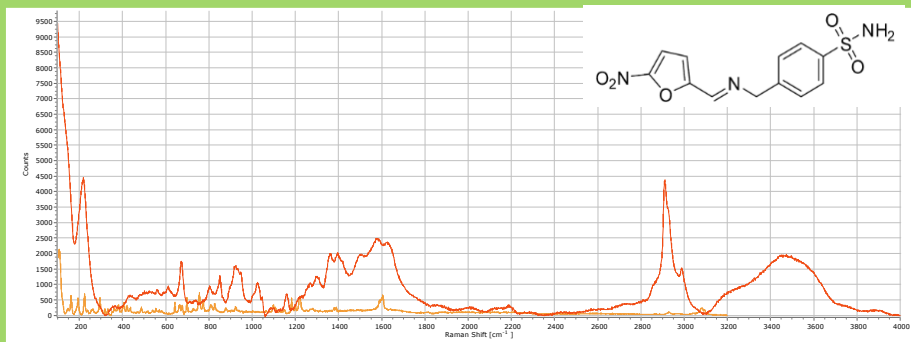
Silver Nanoparticles – TEM images



Sample Analysis



Comparison between Raman and SERS spectra

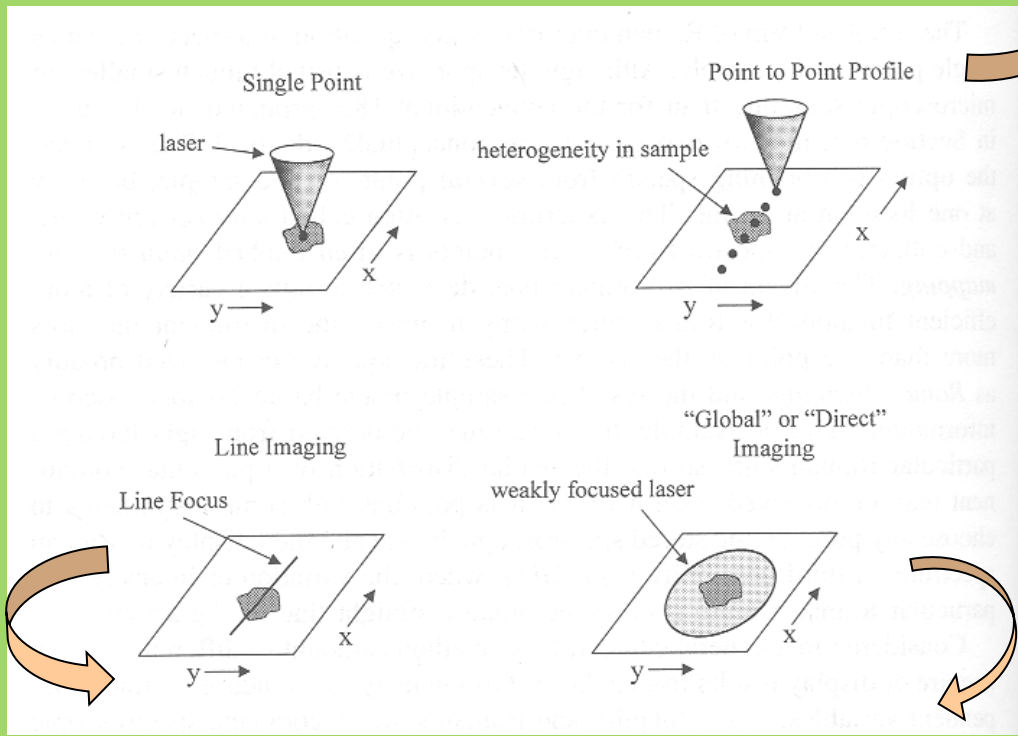


In situ Raman microscopy

Mapping Raman

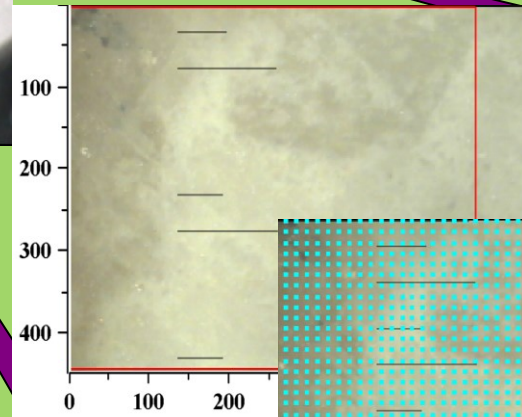
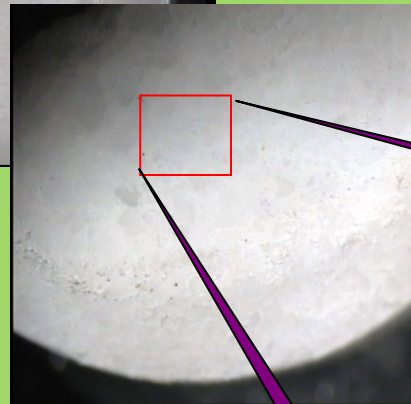
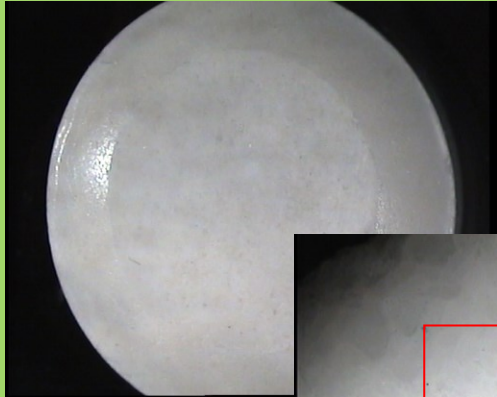
Line Profile
Distinct point
Area

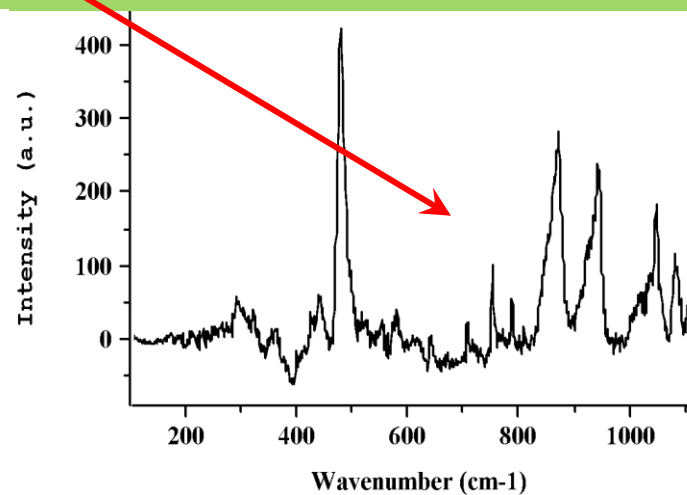
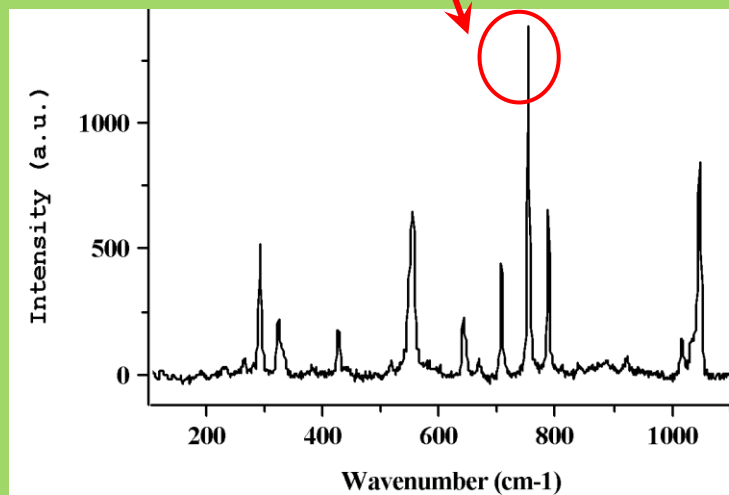
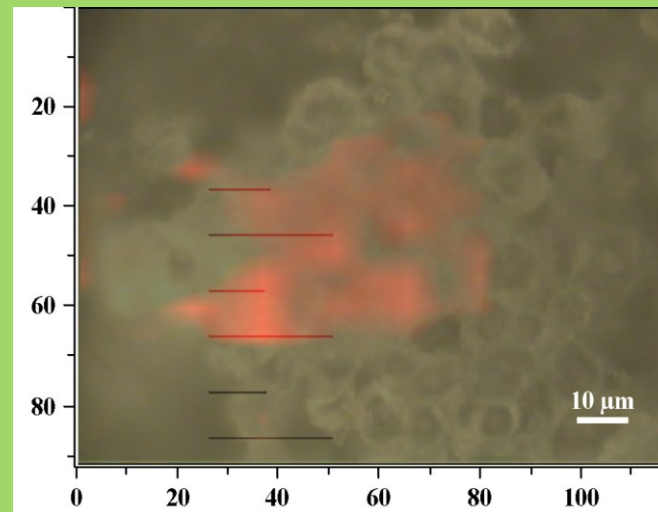
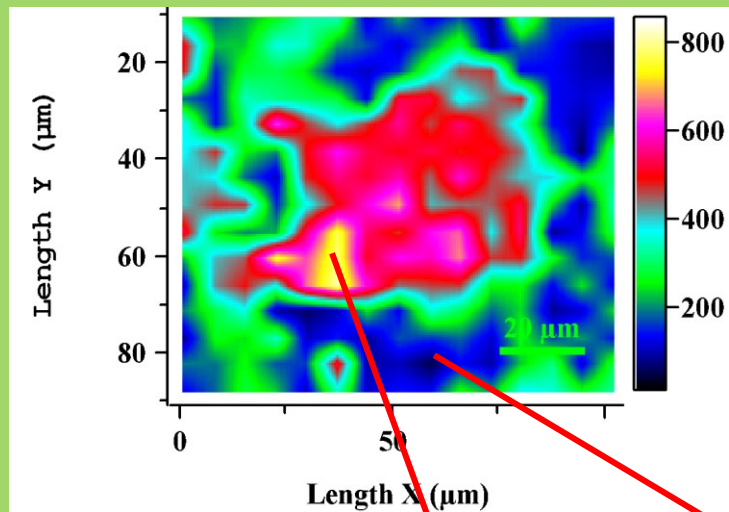
Point to point profile



Line imaging

Direct imaging





New frontier for cells
characterization.
Spectralomics: the
Raman Spectroscopy

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Prof Graziella Pellegrini, UNIMORE
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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

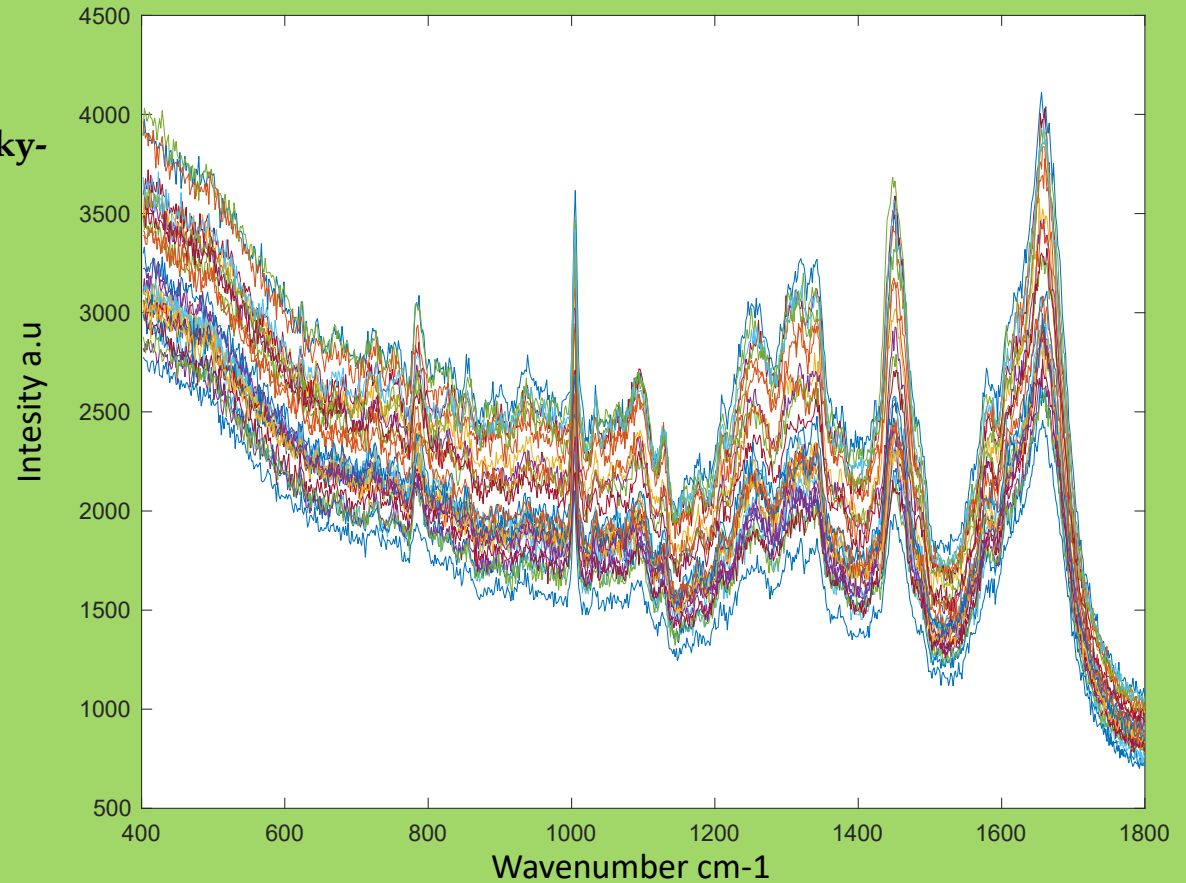
The paradigm of Spectralomics is an approach to **interpretation and representation of cellular events** and processes in terms of **changes to the spectral profiles, compared to controls**, or as a function of time or dose, rather than specific biochemical components or biomarkers.

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. Pre-processing

- ❖ Filtering (polynomial Savitzky-Golay filter)
- ❖ Extended Multiplicative Signal Correction (EMSC)
- ❖ Scaling ('Normalization')



1

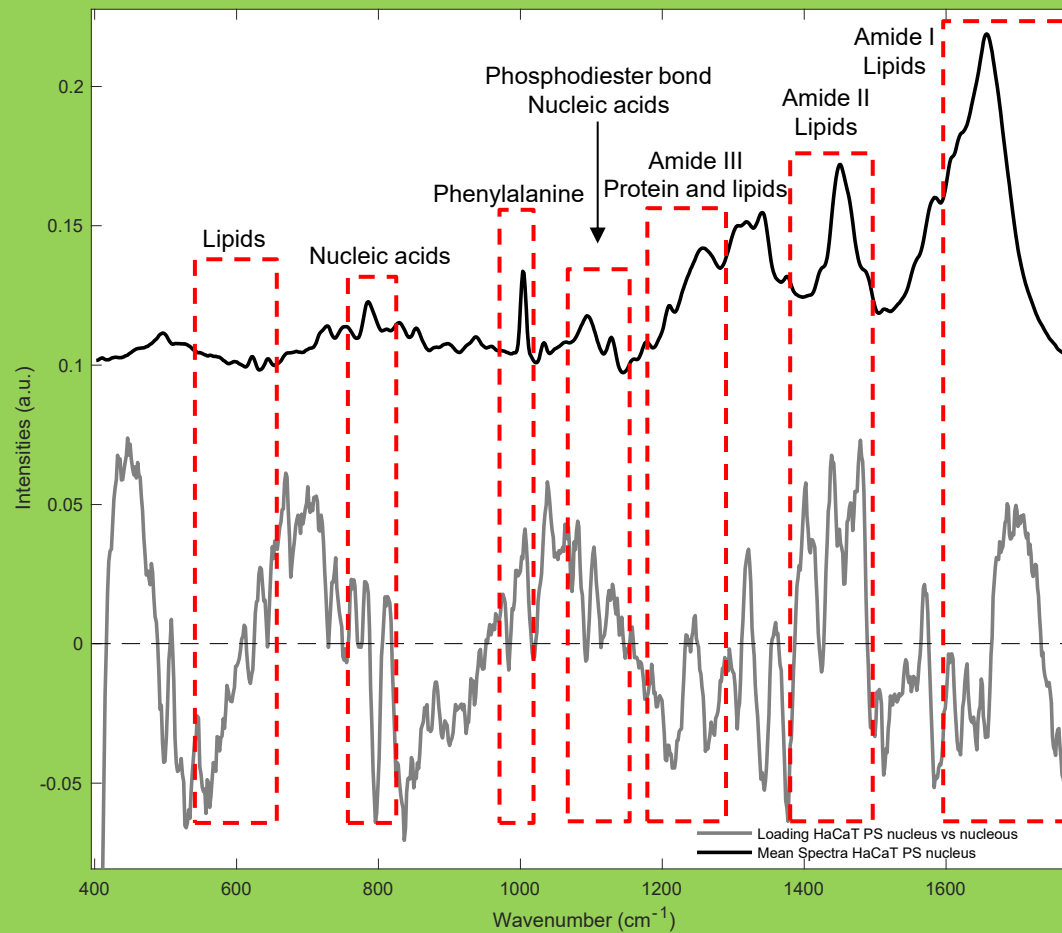
2

3

4

5

6



CHEMICAL ASSIGNMENT

Peaks analysis

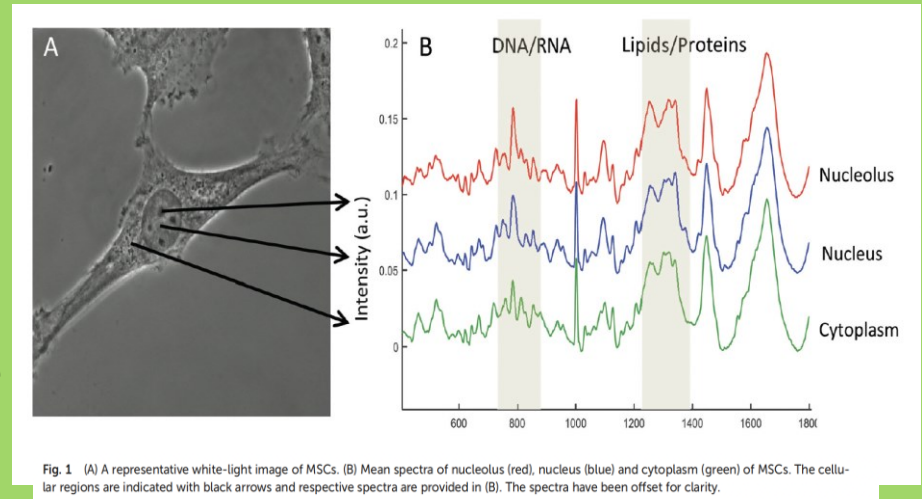
POINT SPECTRA

30 cells each analysed in 3 points:

- Cytoplasm
- Nucleus
- Nucleolus

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

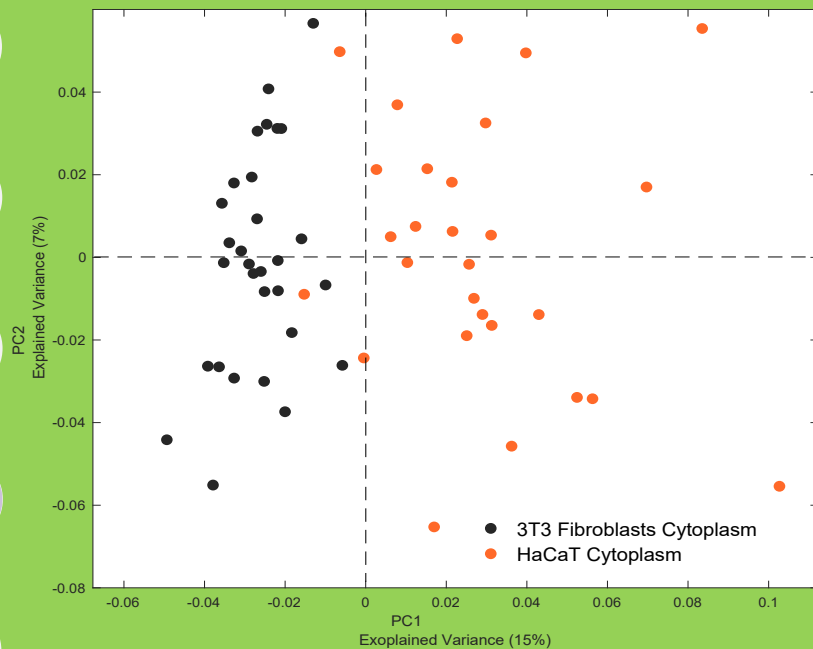
90 spectra x 5 (cell types) = 450 spectra



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

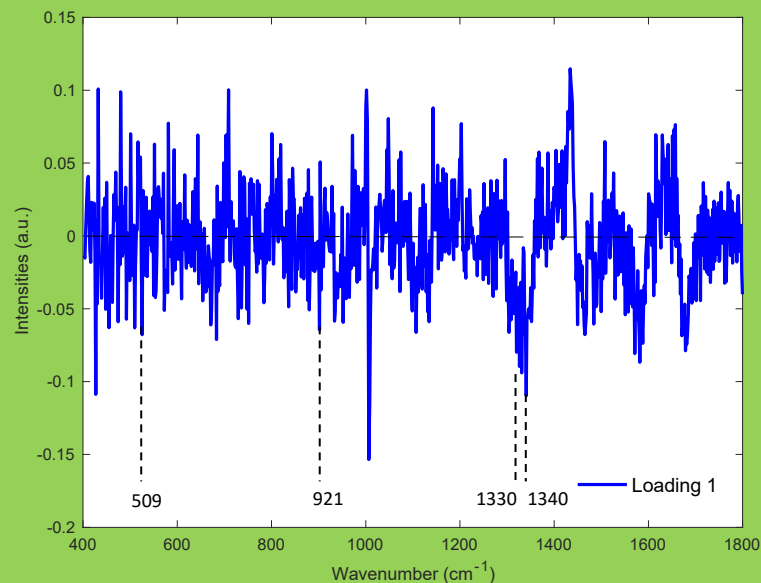
509, 921, 1330, and 1340 cm^{-1} Collagen

519 Phosphatidylserine

1006 Phenylalanine

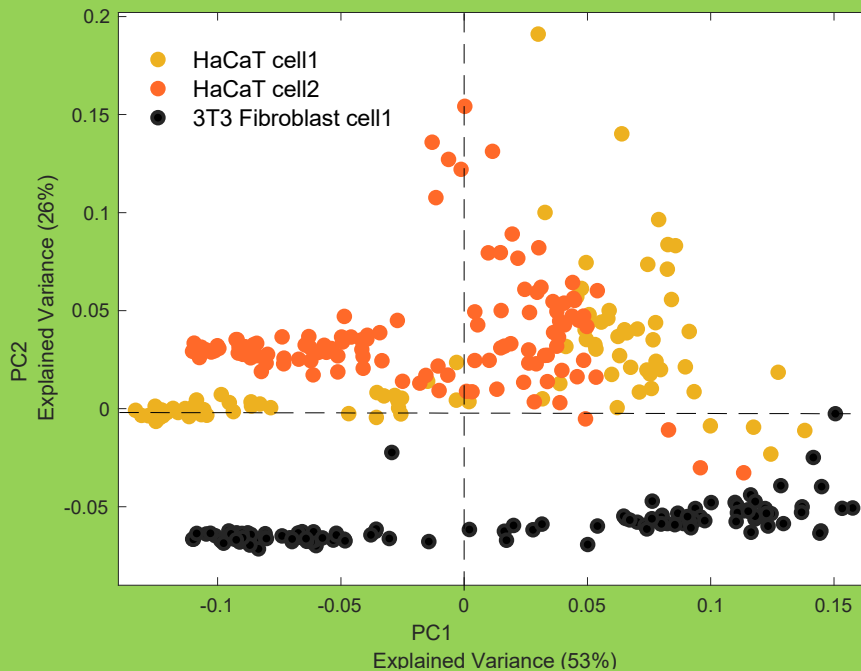
1330 DNA/ phospholipids

1581 Pehylalanine



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

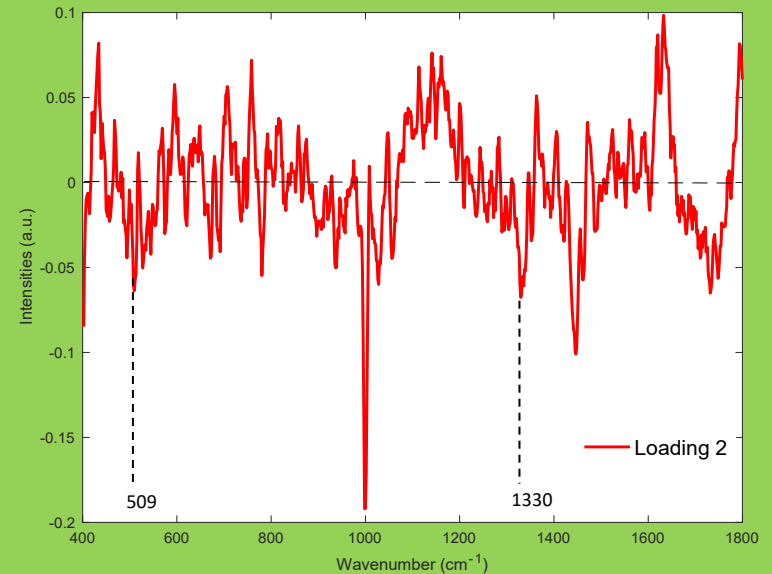
509 cm^{-1} Collagen

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