

Raman spectroscopy

Principle and applications

Sanni Ilmaranta, 2024

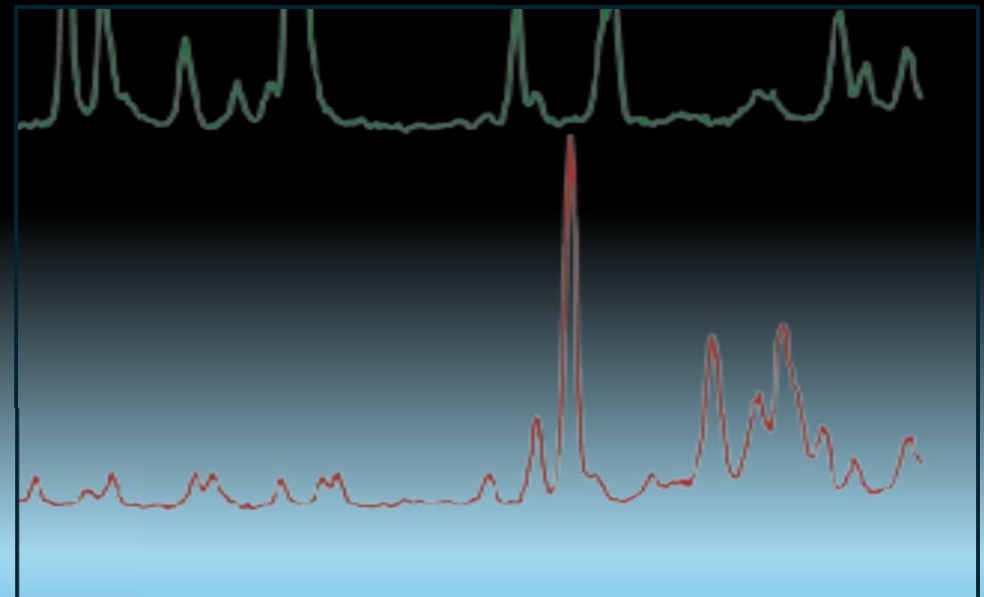
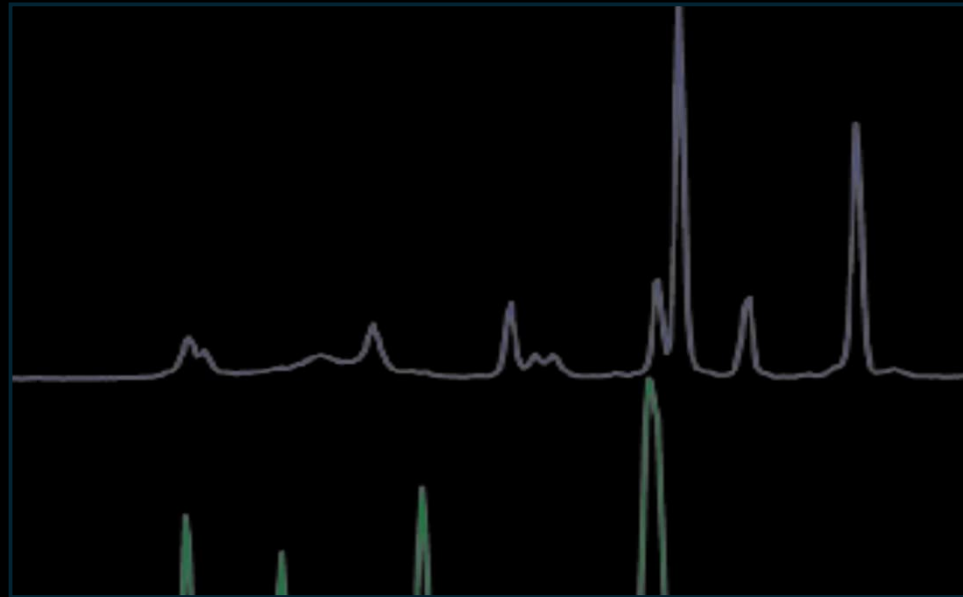


Table of contents

1. History and background

2. Principle and theory

3. Information and data interpretation

4. Applications

5. Pros and cons

6. Current research

- Electronic and Vibrational Properties of TiS_2 , ZrS_2 , and HfS_2 : Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods
- Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

7. Wrap up and summary

1. History and background



- Raman spectroscopy is named after **Sir Chandrasekhara Venkata Raman**, and Indian physicist known for his work in the field of light scattering
- Raman earned the **Nobel Prize for physics in 1930 for this research and work**



- During his voyage to Europe in 1921 Raman started to wonder about the blue colour of glaciers and the Mediterranean sea
- His experiments regarding the **scattering of light** by water and transparent ice blocks resulted in phenomena called the **Raman lines and Raman effect**
- Early experiments were faced with scepticism and results were hard to reproduce, but soon came to be widely recognized

History and background

Early days
1930's to 50's

Excitation: monochromatic light, usually from mercury arc lamp

Separation of wavelengths: Simple prisms or diffraction gratings

Detection: photographic plates or photomultiplier tubes



First commercial Raman instruments

1966→

Lasers and dispersive gratings were introduced to the setup

Raman microscopes and charge coupled devices (CCD) detectors



Modern days

A large scale of different Raman applications from microscopes to cuvette-based systems are available.



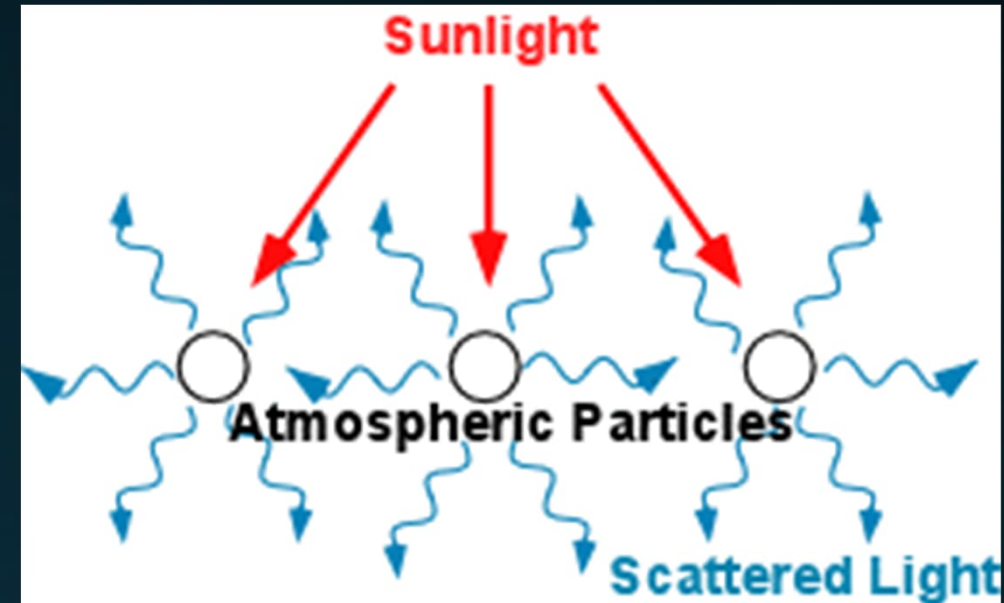
2. Principle and theory

Raman spectroscopy is an analytical technique, where **scattered light** is used to measure **the vibrational energy modes in a sample**

Principle and theory

Scattering of light

- Scattering refers to the way light rays are reflected or dispersed in various directions when they pass through a medium containing particles.
- A change in direction of motion of a particle because of a collision with another particle.
- **Particles in this context can refer to**
 - atoms and molecules
 - boundaries between different mediums
 - crystals
 - irregularities or changes in density and composition of the medium.
- Scattering involves properties from both the radiation light and from the matter it interacts with.
- Scattering can explain the differences in colour and intensity of light in different contexts, such as **blue sky or glaciers**.



Air molecules such as oxygen and nitrogen molecules are small and effective at scattering shorter wavelengths of light (blue and violet).

Scattering of light can be divided in to two main categories:

Rayleigh scattering and Raman scattering

Principle and theory

Rayleigh scattering = elastic scattering

- Light or photons are scattered from the matter with the **same frequency** as the incident light
- Wavelength of the scattered light remains the same as the incident wavelength and the state of the material isn't changed → **preservation of kinetic energy**
- It is the most common type of scattering
- Produced **by small particles** with approximately 1/10 size of the incident wavelength
- Resulting from **polarization of particles**: charge separation when subjected to an external electric field and distortion from normal shape

Principle and theory

Raman scattering = inelastic scattering

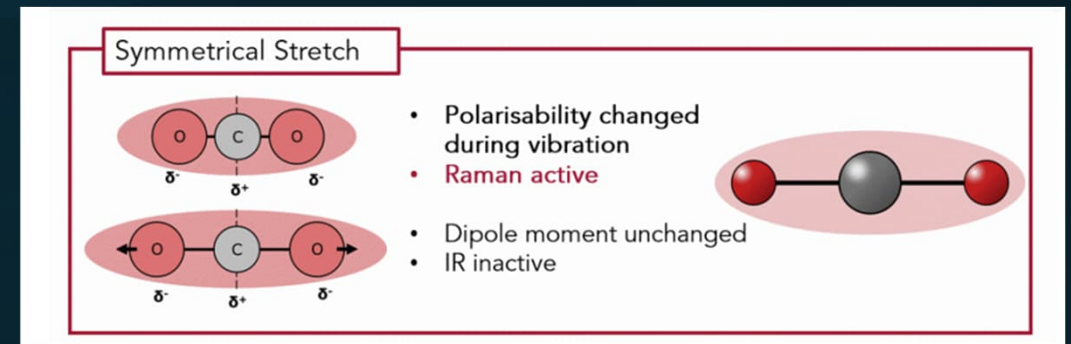
- Light or photons are scattered from the matter with **different frequency** than the incident light
- Upon interaction with a photon, the energy is transferred to a molecule which is excited to a virtual, higher energy level. This state is unstable and short-lived, resulting in a relaxation to a vibrational energy level of a different energy as before
- The energy difference between incident photon and scattered photon is called the **Raman shift**
- Resulting scattering may occur either with
 - 1) Smaller energy and longer wavelength, resulting in **Stokes Raman scattering**. Results when a ground state molecule is excited and relaxes back to a higher energy state. **This is typically measured in Raman spectroscopy.**
 - 2) Higher energy and smaller wavelength, resulting in **Anti-Stokes Raman scattering**. Results when a molecule is already at an excited state, gains energy and relaxes back to ground state.
- Approximately **1 in a 10 million** photons are scattered inelastically, so only a small portion of the total scattering is useful for Raman
- Raman shift can be used as a fingerprint to identify substances
- Raman scattering is due to **polarization** of a molecule in relation to its vibrational motion



A molecule is Raman-active when polarizability changes during vibrational motion

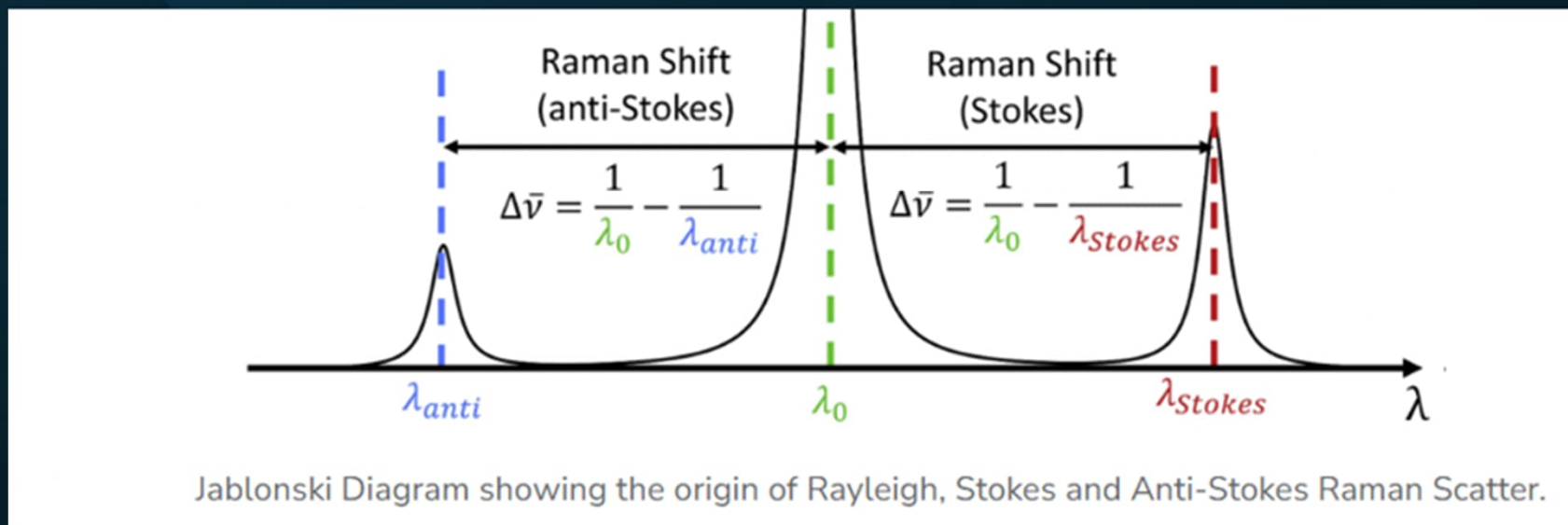
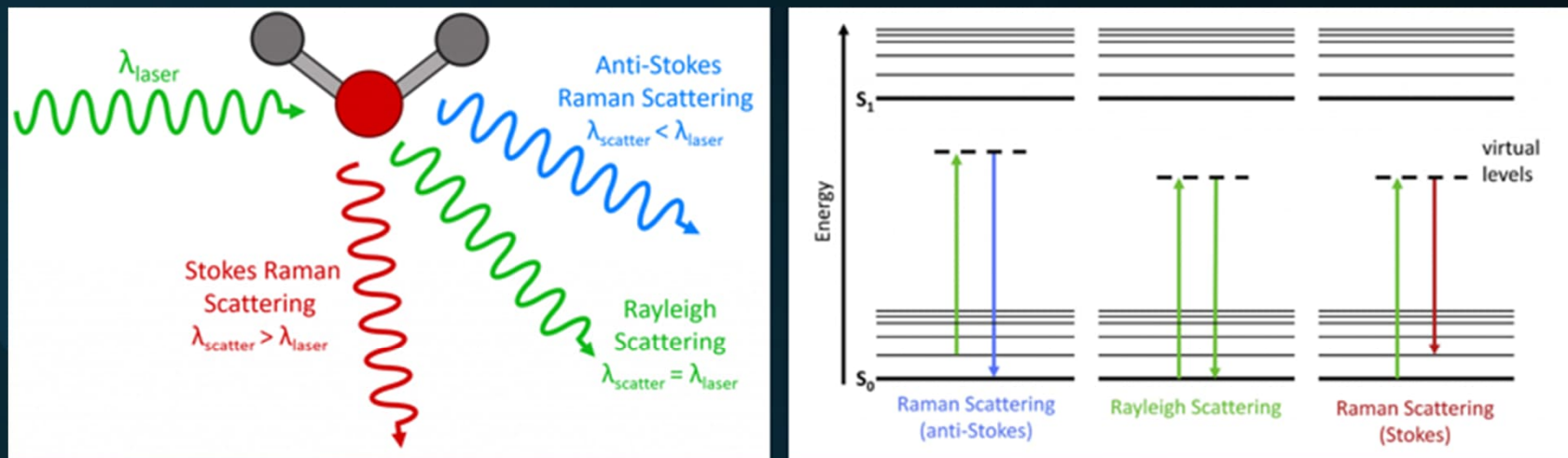


Symmetrical stretch is Raman active



Principle and theory

Rayleigh vs. Raman scattering



Principle and theory

Instrumentation

Raman spectrometers consist of three main components:

1) A high intensity laser is used as an excitation source.

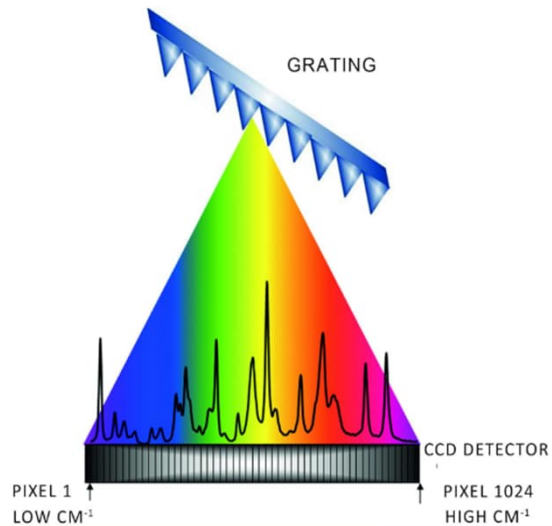
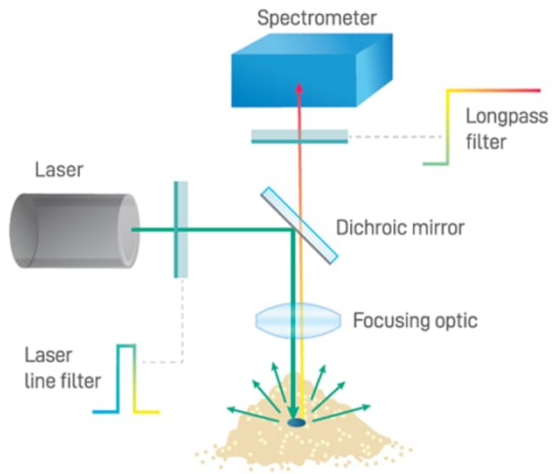
- High intensity is required to achieve the rare Raman scatters
- Scattering intensity is inversely proportional to the excitation wavelength λ^{-4} so shorter laser wavelengths yield better signal and vice versa

2) A sample interface refers to the optics that direct and focus the incident beam and collect the Raman emission to spectrometer.

- Some sort of filters are typically used for either the laser or to block overwhelming Rayleigh scattering

3) Spectrometer is used to capture and detect all the light transmitted by the sample interface.

- Spectrum is reported as a function of Raman shift relative to laser frequency
- CCD detectors a common detector in Raman spectroscopy. They are suitable for UV-VIS and near-infra regions
- CCD detectors are highly sensitive and allow a multichannel operation enabling the detection of the entire Raman spectrum in a single detection



Principle and theory

Instrumentation

- For routine analysis and basic sample identification a very high resolution is typically not needed, and the “basic” Raman spectrometer is used.
- Characterization of e.g. polymorphs and crystallinity on the other hand requires high resolution, which can be obtained with **Raman spectroscope**:
 - A combination of a Raman spectrometer with a standard optical microscope
 - The excitation laser beam is focused through the microscope to create a micro-spot on the sample
 - The Raman signal from the sample is collected and passed back through the microscope into the spectrometer
 - This allows the spectroscopy to be done with a microscopic spatial resolution



- **Combination or hyphenated Raman systems** allow more complete analysis on a single system
- Raman can be coupled with e.g. photoluminescence detection or SEM imaging

3. Information and data interpretation

- Raman spectroscopy is a non-destructive method, where the radiation from a **laser** interacts with molecular vibrations, resulting in an energy shift of scattered light known as the **RAMAN SHIFT**
- This shift in energy provides useful information about **vibrational modes** in the system
- It can be used to identify different types of bonds present in a compound

Information:

- Chemical structure and bonds
- Phase and morphology
- Crystallinity
- Molecular interactions



A Raman spectrum or Raman fingerprint is specific to a particular molecule or material!

Information and data interpretation

- **Raman spectrum** includes peaks with different intensities and positions of Raman scattered light
- Each peak in the spectrum corresponds to a specific molecular bond vibration
- Bonds and bond types between atoms can be detected and identified
- Different spectral libraries are used for identification
- Raman spectroscopy provides both **qualitative and quantitative information**:
 - general spectrum profile with peak positions and relative peak intensities can be used for qualitative identification
 - intensity for each peak is directly proportional to concentration and quantitative analysis can be done based on calibration samples with known concentrations
 - relative and absolute peak intensity can be used to gain information from mixtures

Information and data interpretation

Suitable **samples** to be analysed with Raman spectroscopy include

- Solids, powders, liquids gels, slurries and gases
- Inorganic, organic and biological material
- Pure chemicals, mixtures and solutions
- Metallic oxides and corrosion

Metals and their alloys **are not** suitable for Raman analysis!

In general, Raman spectra can be obtained for nearly all samples that contain true molecular bonds

Information and data interpretation

- A wavenumber cm^{-1} , a reciprocal of the wavelength is used in Raman spectra.
- It is related to energy and thus to the energy differences between molecular vibrational levels
- The energy of the laser is defined as the zero wavenumber instead of laser wavelength, which does not play a role in the result anymore.
- This means that any laser wavelength can be used for excitation and the Raman shift will always be the same for same vibrational frequencies in the spectra.
- This makes the comparison and spectral interpretation easy and reproducible.



Identification based on spectral fingerprint



Concentration or amount of a material in sample

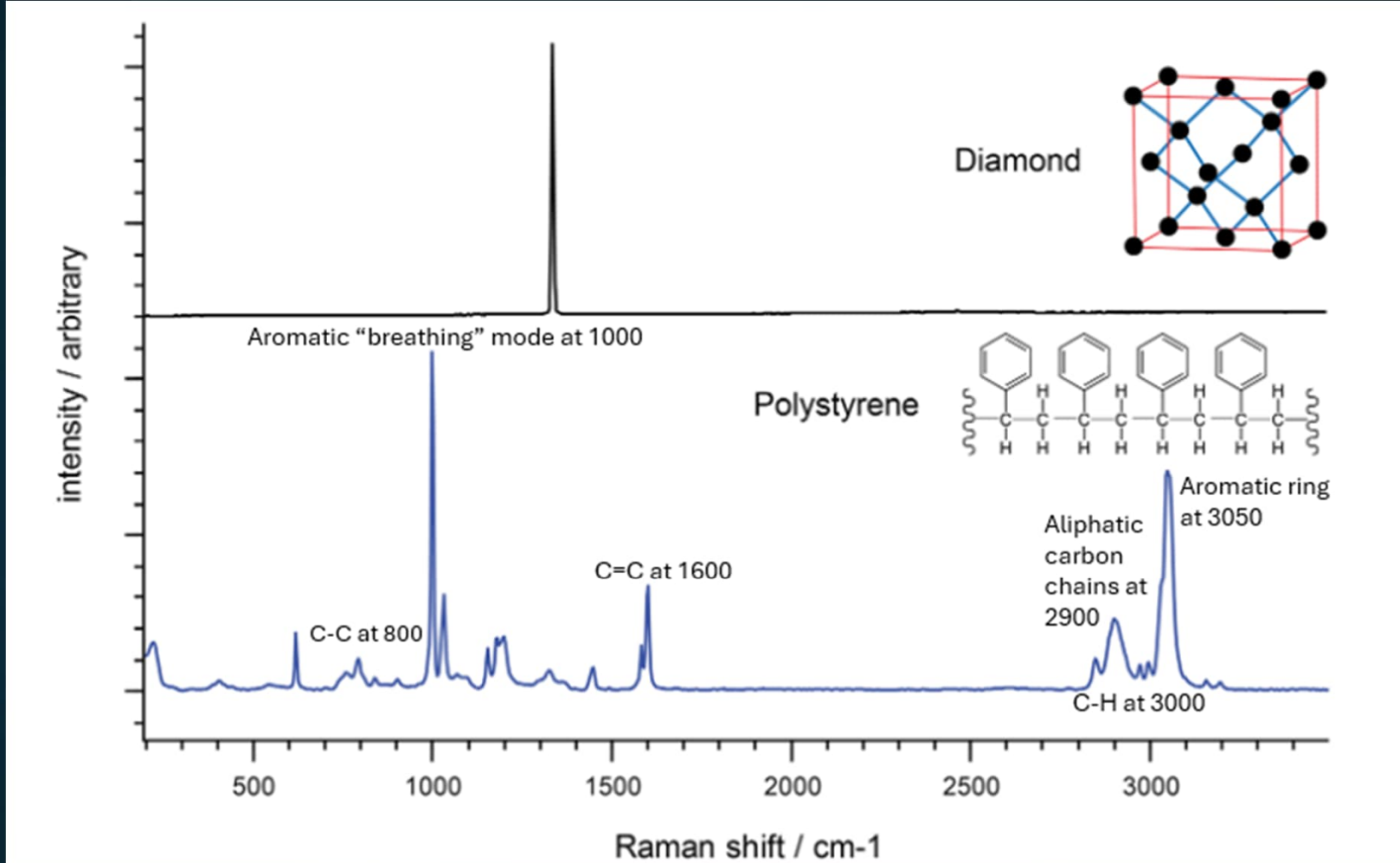


Variation in band position gives information of compressive or tensile stress



Variation in band width gives information of uniformity of bond lengths and crystallinity

Information and data interpretation



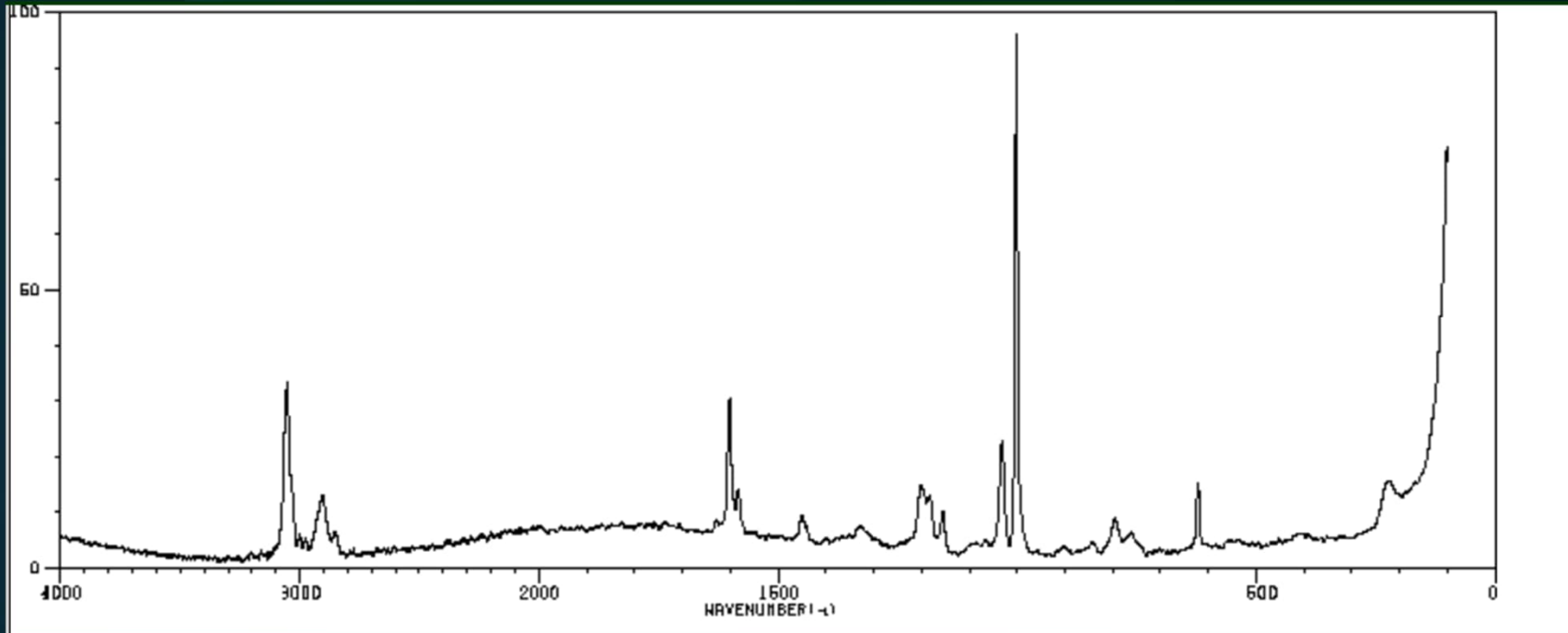
Raman spectra can be interpreted in terms of different functional groups in the sample.

Diamond contains carbons in a regular tetrahedral network, typically resulting in only one dominant band.

Polystyrene is more asymmetric and contains different atoms and different bonds, producing a more complex spectrum.

Heavier atoms and weak bonds have lower Raman shifts than light atoms and strong bonds.

Information and data interpretation



A spectrum for polystyrene, obtained from AIST Spectral database for organic compounds SDBS

Raman spectra gets complex quite fast, so instead of manual work, spectral libraries are used.

Typically, the fingerprint region of $300 - 1900 \text{ cm}^{-1}$ is used for identification.

4. Applications

Raman spectroscopy is used in many fields, especially when **non-destructive, microscopic, chemical analysis and imaging is needed**

Pharmaceuticals and cosmetics

- Raw material verification
- Crystallinity
- Powder content and purity

Geology and mineralogy

- Phase transitions
- Gemstone and mineral identification

Semiconductors

- Purity and contamination identification
- Defect analysis
- Doping effects
- Hetero-structures
- Superlattice structures

Carbon materials

- Purity of carbon nanotubes and their electrical properties
- Hybridization structures in carbon materials
- Diamond like coating properties
- Electrical properties and number of layers of graphene

Life sciences

- DNA/RNA analysis
- Metabolic accretions
- Disease diagnosis
- Characterization of biomolecules

5. Pros and cons

Pros:

- Can be used to analyse a variety of different samples (solids, liquids, gases)
- Little to no sample preparation is needed
- Non-destructive
- No expensive vacuum equipment required
- Analysis time is fast
- Suitable for aqueous samples without H₂O interference

Cons:

- Cannot be used for metals or alloys
- Raman effect is typically weak, leading to low intensities making low concentration measurements difficult.
- Fluorescence from certain materials can cause interference

6. Current research

THE JOURNAL OF
PHYSICAL CHEMISTRY C

Cite This: *J. Phys. Chem. C* 2018, 122, 26835–26844

Article

pubs.acs.org/JPCC

Electronic and Vibrational Properties of TiS_2 , ZrS_2 , and HfS_2 : Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

Nina Glebko, Irina Aleksandrova, Girish C. Tewari, Tripurari S. Tripathi, Maarit Karppinen,^{id} and Antti J. Karttunen*^{id}

Department of Chemistry and Materials Science, Aalto University, P.O. Box 16100, FI-00076 Aalto, Finland

Current research

Electronic and Vibrational Properties of TiS₂, ZrS₂, and HfS₂: Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

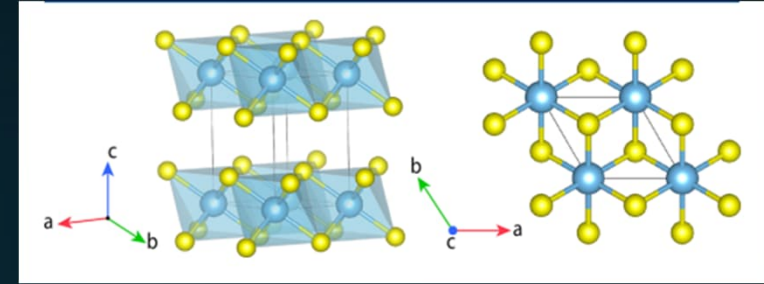
- The study was focused on group 4 metal disulfides TiS₂, ZrS₂ and HfS₂
 - Periodic trends in electronic band structures
 - Electronic transport coefficients
 - IR and Raman spectra
 - Phonon dispersion properties
- The periodic trends in electronic, vibrational and thermoelectric properties were studied by applying a dispersion-corrected hybrid density functional methods and Gaussian-type local basis sets to describe both the crystal structure and electronic structure in all the three materials
- Comparison to available experimental data
- Transport and vibrational properties of thin TiS₂ single crystals were also investigated experimentally

Current research

Electronic and Vibrational Properties of TiS₂, ZrS₂, and HfS₂: Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

Background

- The layered structure of group 4 transition metal disulfides allows as vast number of possible intercalation reactions where guest atoms or molecules are introduced in the interlayer space
- This is an interesting subject, because tuning the parent lattice can yield interesting new properties in the material
 - lower thermal conductivity
 - higher thermoelectric efficiency
- The fundamental electronic properties of these group 4 disulfides have been investigated in several publications and are known to be n-type semiconductors
- These materials are potential thermo electric materials due to their performance improvements arising from the intercalations



General crystal structure for the studied MS₂ compounds (M=Ti, Zr, Hf). Left: Side view. Right: top view. Blue: M, yellow: S

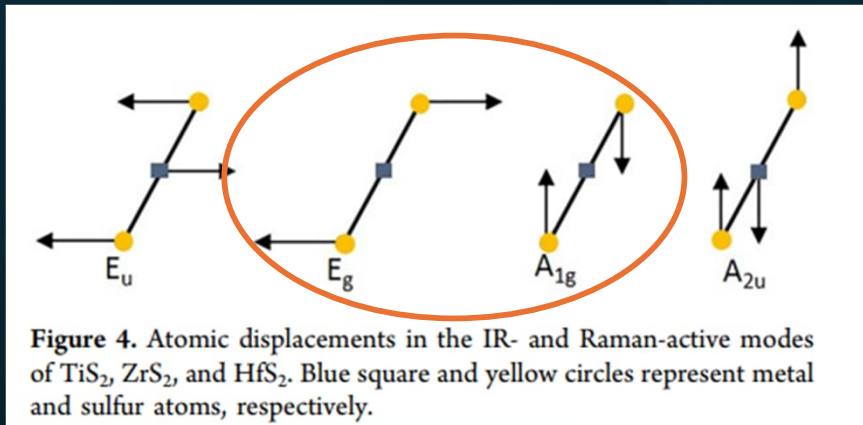
- TEs are materials used to convert temperature differences directly into electrical voltage or energy and vice versa.
- They are used in different applications such as
 - industrial settings
 - microelectronics
 - human body/ambient air interface

Cheaper and more environmentally friendly TE materials are needed to substitute the more commonly used materials like Bi₂Te₃ or PbTe_{1,2}

Current research

Electronic and Vibrational Properties of TiS₂, ZrS₂, and HfS₂: Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

- DTF calculations and simulations (including IR and Raman) done with CRYSTAL14 program
- Experimental section included the synthesis of single crystal TiS₂ via vapor transport method
- IR and Raman spectra were obtained
- Interplane Seebeck coefficient, DC electrical resistivity and Hall coefficient with carrier concentration were measured using the Quantum Physical Property Measurement System



- Vibrational properties of a material can be used in explaining various thermodynamic and transport properties of solids, including **lattice thermal conductivity**
- Primitive cells of TiS₂, ZrS₂ and HfS₂ consists of two chalcogen atoms and one metal atom with trigonal prismatic coordination
- This results in three acoustic modes (related to the overall movement of atoms in the lattice) and six optical modes (related to the relative moments between atoms in the lattice)
- Optical modes are further split into three g-modes (symmetric) and three u-modes (asymmetric) due to inversion centre

Current research

Electronic and Vibrational Properties of TiS₂, ZrS₂, and HfS₂: Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

Table 2. Calculated Vibrational Frequencies of the IR and Raman Active Optical Modes in TiS₂, ZrS₂, and HfS₂ (cm⁻¹ units)^a

mode	TiS ₂	ZrS ₂	HfS ₂
E _u	115 (-35.8%)	168 (-6.4%)	169 (+1.6%)
E _g	258 (+9.8%)	263 (+4.7%)	270 (+3.2%)
A _{1g}	361 (+7.8%)	340 (-2.7%)	346 (+2.6%)
A _{2u}	408 (+9.8%)	346 (-1.2%)	319 (-1.9%)

^aThe values in parentheses show the difference in the experimental spectra.^{60,61}

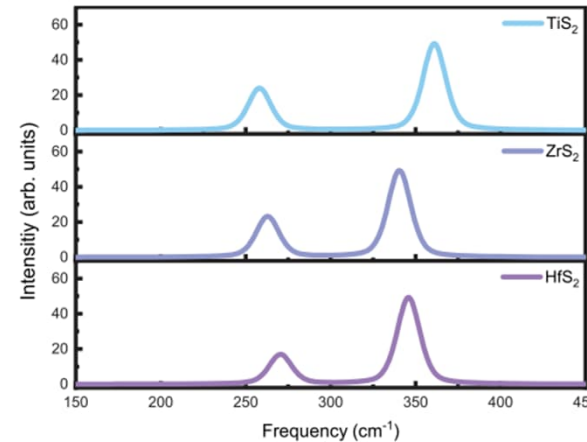


Figure 5. Calculated Raman spectra for TiS₂, ZrS₂, and HfS₂.

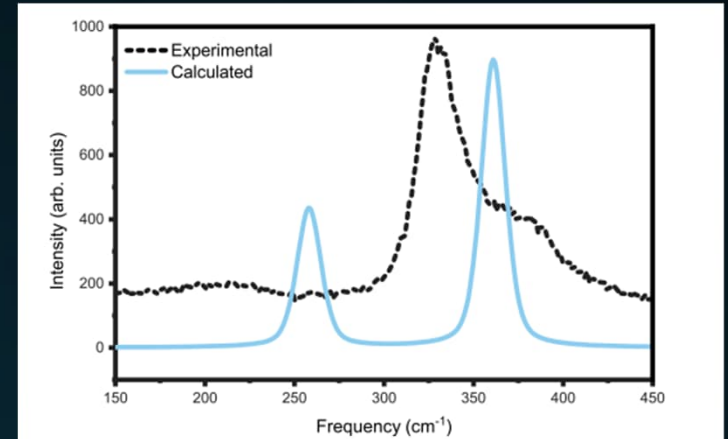


Figure 7. Simulated and experimental Raman spectrum of TiS₂ (both spectra from this work).

- For ZrS₂ and HfS₂ the calculated vibrational values are in line with experimental values. For TiS₂ the difference between theory and experiment is large.
 - no clear conclusions why, but could be related to the tendency for non-stoichiometry in TiS₂ single crystals
- The broadening of the peak and appearance of the shoulder for all the three group 4 disulfides has been detected in other studied too - smaller on and the other side for HfS₂ and ZrS₂.
 - Possibly due to coupling of IR and Raman active modes from resonance effect or non-stoichiometry
 - The shift to the smaller wavelength and shouldering compared to the calculated model can be due to increasing carrier concentration in TiS₂.

Current research

Electronic and Vibrational Properties of TiS₂, ZrS₂, and HfS₂: Periodic Trends Studied by Dispersion-Corrected Hybrid Density Functional Methods

Conclusions:

- The developed DFT model was well suited for investigation of layered transition metal dichalcogenide materials with weak interlayer van der Waals interactions.
- Systematic investigation of the periodic trends within group 4 disulfides revealed that TiS₂ showed many differences to ZrS₂ and HfS₂ due to more covalent M-S bonding in TiS₂.
- ZrS₂ and HfS₂ mainly showed differences for properties where the atomic mass played a role
- All three compounds showed similar Seebeck coefficients (similar physical properties that quantify the magnitude of electromotive force or voltage generated when material is subjected to a temperature gradient)
- Clear differences in the relative electrical conductivity of cross- and in-plane directions

Current research

Sensors & Actuators: B. Chemical 395 (2023) 134497



ELSEVIER

Contents lists available at [ScienceDirect](#)

Sensors and Actuators: B. Chemical

journal homepage: www.elsevier.com/locate/snb



Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

Luyue Jiang^{a,1}, Matthew Xinhu Ren^{b,1}, Gang Niu^{a,g,*}, Jingang Shi^{c,**}, Xinhao Cao^a, Yan Duan^d, Heping Wu^{a,f}, Zhen Xie^{d,**}, Yi Quan^e, Libo Zhao^{f,g}, Zhuangde Jiang^g, Yihong Gong^c, Wei Ren^{a,g,*}, Gang Zhao^{d,**}

^a Electronic Materials Research Laboratory, Key Laboratory of the Ministry of Education & International Center for Dielectric Research, School of Electronic Science and Engineering, Xi'an Jiaotong University, Xi'an, China

^b Biology Program, Faculty of Science, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada

^c College of Software Engineering, Xi'an Jiaotong University, Xi'an 710049, China

^d The College of Life Sciences and Medicine, Northwest University, Xi'an, Shaanxi 710069, China

^e School of Microelectronics, Xidian University, Xi'an 710071, China

^f School of Instrument Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China

^g The State Key Laboratory for Manufacturing Systems Engineering & The International Joint Laboratory for Micro/Nano Manufacturing and Measurement Technology, Xi'an Jiaotong University, Xi'an 710049, China



Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

- The study was focused on achieving a rapid, accurate and label-free identification and categorization of four important leukemia cell lines to a sub-class level using Raman spectroscopy and identification model.
- Different leukemia cells were grown on a gold film-covered glass substrate to ensure good morphology and high-quality Raman spectra
- Even though the studied cell lines were similar in shape, they each produce a different spectra
- The developed method could be used by doctors for early diagnosis of leukemia and to determine treatment plans faster and to improve the diagnosis of patients.

Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

Background

- Raman spectroscopy can obtain the biochemical characteristics of both cells and tissues
- Characteristic fingerprint can be obtained without conjugated antibodies, dyes or radioactive labels.
- Four cell lines **K562**, **U937**, **Raji B** and **Jurkat** were identified and sorted with statistical methods and analysis based on Raman data.
- Jurkat and Raji B cells are relatively mature and commonly used acute lymphoblastic leukemia subclass models
- A material choice for the substrate is critical, and gold-coating is a cheap and easy alternative - gold particles possibly enhancing Raman effect
- Current clinical diagnosis for leukemia is based on the manual detection of blood cells via recognition of morphological features of stained cells
- Many leukemia cells are similar in morphology and these tests suffer of poor sensitivity and slow detection rate, resulting in prolonged treatment times.

Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

- Cells were deposited on a gold film covered glass slide and analysed with a **micro-Raman spectrometer**
- First erythrocytes (red blood cells) and three typical leukemia cells were identified and sorted with statistical methods and then acute lymphocytic leukemia Raji B (for B-cell line) and Jurkat (for T-cell line) were categorized using Raman data
- **After statistical analysis and methods based on the obtained Raman spectra, data was classified into four categories and two subclasses.**
- Surface morphology was measured with optical microscopes and scanning electron microscope SEM.
- A finite difference time domain FDTD simulation was conducted to study the electromagnetic field distribution of gold nanoparticles in the gold-coated glass substrate.

Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

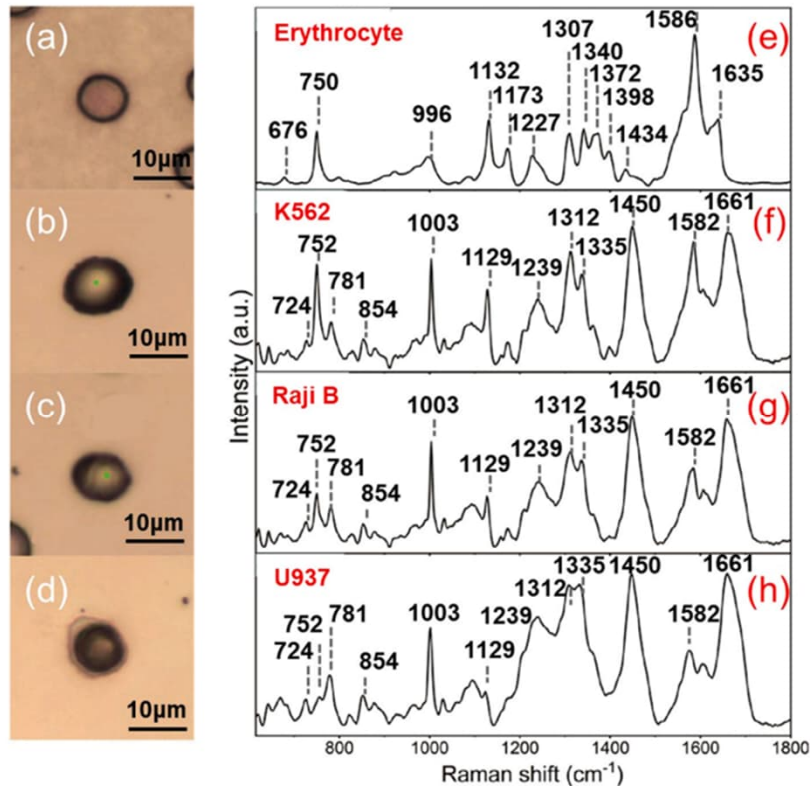


Fig. 2. (a, b, c and d) Optical microscope (OM) image and (e, f, g and h) Raman spectra of erythrocytes, K562 cells, Raji B cells, and U937 cells, respectively on the gold film covered glass slide.

- Based on optical microscopy and morphology, it is very difficult to differentiate the red blood cells from leukemia cells and latter from each other
- It is clear, the spectra for erythrocyte/red blood cell is very different from those of the three leukemia cell lines
- The three leukemia cells have similar spectra, that differ in intensities

Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

Sensors and Actuators: B. Chemical 395 (2023) 134497

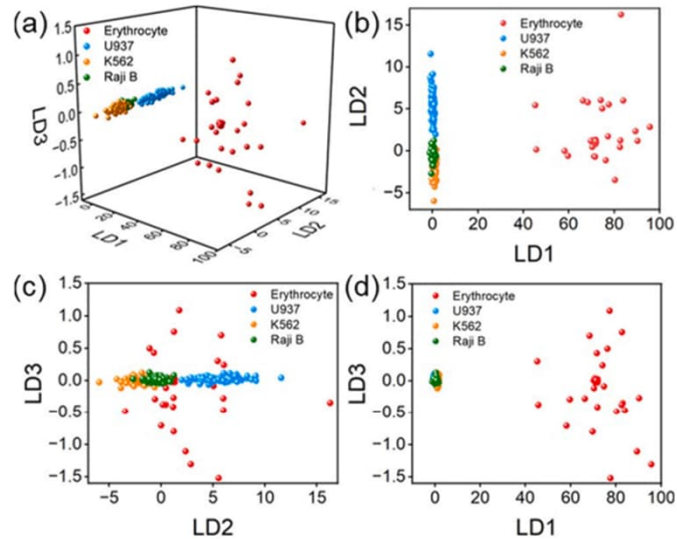


Fig. 3. (a) LDA-KNN three-dimensional scatter plot, (b) Scatter plot of LD1 and LD2, (c) Scatter plot of LD2 and LD3, and (d) Scatter plot of LD1 and LD3 for 320 spectra obtained from erythrocyte (red balls), U937 (blue balls), K562 (orange balls), and Raji B (green balls).

L. Jiang et al.

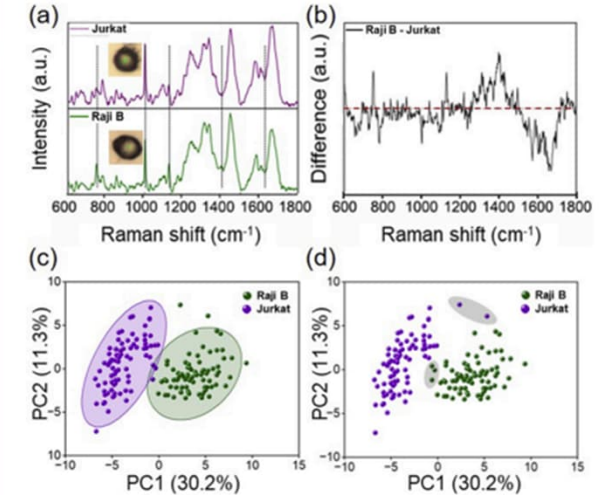
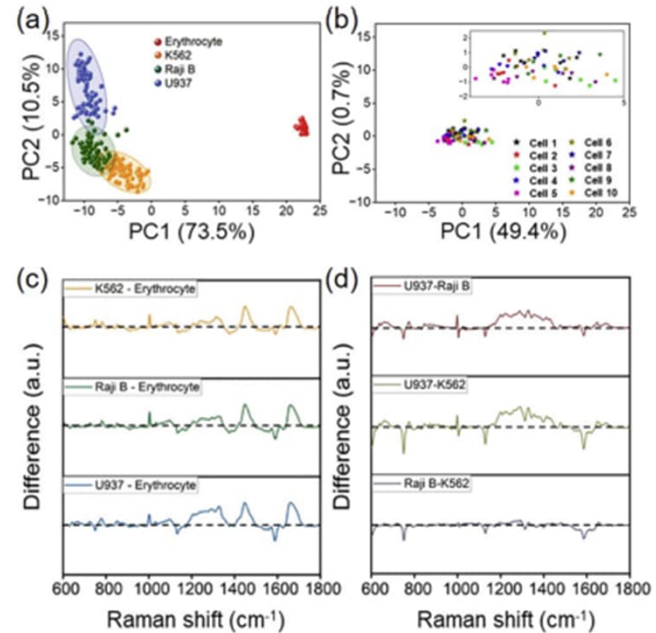


Fig. 5. (a) Average Raman spectrum of Raji B (green line) and Jurkat cells (purple line); (b) The difference spectra obtained by subtracting Jurkat from Raji B cells; (c) Scatter plot of unsupervised identification model (PCA-KMCA) constructed using 160 spectra from Raji B (green balls), and Jurkat cells (purple balls). The shaded areas are 95% confidence ovals for the corresponding cell type; (d) PCA score plot of PC1 and PC2 for 160 spectra from Raji B cells (green balls), and Jurkat cells (purple balls). The shaded area in gray is the area of KMCA misidentification.

- The spectral differences between the two acute lymphoblastic leukemia subclass cell lines were smaller than the differences between the different classes of leukemia.

Current research

Label-free, rapid and highly accurate identification and categorization of leukemia cells via Raman spectroscopy

Conclusions:

- The gold-coated substrate was used successfully and effectively addressed the issue of marker band overlap between the glass slide and the proteome Raman peaks.
- Substrate also ensured the intact and good morphology of the cells and the high-quality Raman spectra
- The Raman enhancement factor of gold film covered glass slides was estimated to be 10^4 by using Finite-Difference Time-Domain (FDTD) simulation
- **Statistical analysis:**
 - LDA and KNN supervised identification model was used to analyze the Raman spectra of erythrocytes, K562, U937 and Raji B cells, showing distinct separate clusters with an accuracy of 90.62%
 - Based on the differences in Raman spectra of the four type cell lines, the unsupervised identification model (PCA-KMCA) could automatically form four cluster and the sensitivity, specificity and accuracy of the unsupervised identification model was 93.75%, 96.67%, and 97.50%, respectively
 - In addition, the unsupervised identification using PCA plus KMCA of Raji B and Jurkat cells can be achieved with a sensitivity, specificity and accuracy of 96.25%, 97.50% and 96.88 % respectively.
- **The results demonstrate that Raman spectroscopy can be used to achieve a rapid and accurate identification of acute and chronic leukemia cells and differentiate between lymphoblastic leukemia subclass cells**

7. Wrap up and summary

- Raman spectroscopy is based on inelastic Raman scattering and it measures the vibrational energy modes and a change in polarizability in a molecule
- It is non-destructive and suitable for many different sample types and matrices, excluding metals and alloys
- It is utilized in many different fields from material sciences to life sciences
- Raman spectroscopy is often compared to IR spectroscopy
 - Both are based on molecular vibrations
 - IR is based on absorption of light energy corresponding to the vibrational energy of a molecule and a change in the dipole moment
 - Raman is a scattering technique where the dipole moment stays the same

Thank you!

Questions, comments and
observations are welcome.