Advanced Spectroscopic Techniques: From Raman Scattering to Multi-Dimensional NMR

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Abstract:
Vibrational and magnetic resonance spectroscopies together form the analytical backbone of modern structural chemistry, materials science, and biomolecular research. This review surveys two of the most information-rich spectroscopic families in current use: Raman scattering, from its spontaneous form through enhanced modalities such as surface-enhanced, resonance, tip-enhanced, and coherent Raman scattering; and multi-dimensional nuclear magnetic resonance (NMR), spanning two-dimensional homonuclear experiments such as correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) to heteronuclear experiments such as heteronuclear single-quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC), and their extension into three- and four-dimensional protein NMR. The physical basis, instrumentation, and characteristic spectral signatures of each technique are described, supported by comparative data tables drawn from the peer-reviewed literature. Representative applications are discussed across materials characterization, two-dimensional nanomaterials, biomedical diagnostics, protein structure determination, and metabolomics. A comparative analysis is presented to illustrate the complementary strengths of both analytical methods for the elucidation of molecular structure, dynamics and composition and new directions such as machine-learning-assisted structure elucidation from Raman spectra and hyphenation of Raman with various other instruments such as multi-dimensional NMR are discussed. The review is intended as a practical reference for researchers seeking to select or combine these techniques for structural and analytical investigations.

Keywords: Raman spectroscopy; surface-enhanced Raman spectroscopy; nuclear magnetic resonance; two-dimensional NMR; HSQC; NOESY; vibrational spectroscopy; structure elucidation

1. Introduction

Molecular spectroscopy occupies a central position in the analytical sciences because it converts the quantum-mechanical structure of matter into measurable, interpretable signals. Among the many spectroscopic modalities available to chemists, materials scientists, and structural biologists, Raman scattering and nuclear magnetic resonance (NMR) spectroscopy stand out for the depth and specificity of the structural information they provide. Both techniques are non-destructive, applicable to solids, liquids, and gases, and capable of probing samples ranging from small organic molecules to large biomacromolecules and inorganic solids.

Raman spectroscopy is based on the differential scattering of monochromatic light by molecular vibrations, creating what is known as a "fingerprint" which is characteristic of bond composition, crystallinity, strain, and molecular symmetry (Larkin, 2017). The technique is also very suitable for aqueous environments and has proved to be indispensable in polymer science, semiconductor metrology, pharmaceutical quality control, art conservation and, increasingly, clinical diagnostics (Ranasinghe et al., 2023). To overcome the intrinsic

limitations of spontaneous Raman, a family of enhancement methods has been proposed, such as surface enhanced Raman spectroscopy (SERS), resonance Raman, tip-enhanced Raman spectroscopy (TERS) and nonlinear methods, coherent anti-Stokes Raman (CARS) and stimulated Raman (SRS) spectroscopy (Kudelski, 2019).

In turn, NMR spectroscopy is based on the response of magnetic nuclei to external magnetic field and a sequence of RF pulses. Although one-dimensional NMR has proven useful in identifying a vast number of molecules, it is often not enough to distinguish the resonances of all but the simplest molecules because the chemical shifts are small compared to the number of different nuclei in a typical organic or biological molecule. Multi-dimensional NMR overcomes this limitation by spreading resonances across two, three, or more frequency axes, revealing through-bond and through-space connectivities that are essential for assigning complex spectra and determining three-dimensional molecular structure (Claridge, 2016; Wüthrich, 1986).

The two technique families also differ markedly in the practical demands they place on a laboratory. Raman instrumentation can be benchtop-sized, fibre-coupled, or even handheld, and modern systems require only milliwatts of laser power and seconds of acquisition time for many samples, which has driven adoption well beyond specialist spectroscopy laboratories into process analytical technology, forensic screening, and point-of-care diagnostics. Multi-dimensional NMR, by contrast, depends on superconducting magnets, cryogenically cooled probes, and, for biomolecular applications, isotopically enriched samples, and a single structure determination may require weeks of instrument time. Understanding when each technique, or their combination, is the appropriate choice is therefore as much a practical question as a physical one, and this review aims to make that choice more transparent by placing the two technique families side by side.

This review provides an integrated account of both spectroscopic families. Sections 2 and 3 describe the physical basis, instrumentation, and enhancement strategies of Raman spectroscopy. Section 4 surveys representative applications. Sections 5 and 6 address the fundamentals of NMR and the principal multi-dimensional experiments in current use, followed by their applications in Section 7. Section 8 offers a comparative analysis of the two technique families, Section 9 discusses emerging trends, and Section 10 concludes with an outlook on future directions.

2. Fundamentals of Raman Scattering

2.1 Physical Basis of the Raman Effect

When monochromatic light interacts with a molecule, the great majority of photons are elastically scattered without any change in energy, a process known as Rayleigh scattering. A small fraction, approximately one in every 10^6 to 10^8 photons, is scattered inelastically: the incident photon exchanges energy with a vibrational (or, more rarely, rotational) mode of the molecule (Smith, 2019 in Fan et al., 2019). If the molecule gains vibrational energy, the scattered photon is red-shifted relative to the incident photon; this is termed Stokes Raman scattering. If the molecule loses vibrational energy from an already thermally populated excited vibrational level, the scattered photon is blue-shifted, producing anti-Stokes Raman scattering. Because the population of excited vibrational states follows the Boltzmann distribution, Stokes lines are more intense than anti-Stokes lines at room temperature, and Stokes-shifted Raman spectra are used almost universally for structural analysis.

Figure 1. Energy-level diagram illustrating Rayleigh, Stokes, and anti-Stokes Raman scattering

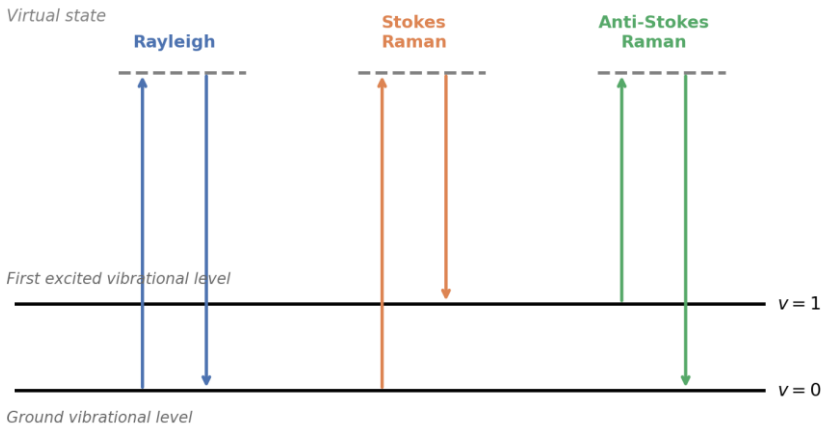

Figure 1. Energy-level diagram illustrating Rayleigh, Stokes, and anti-Stokes Raman scattering. Adapted from the general treatment in Larkin (2017) and Fan et al. (2019).

The Raman effect is fundamentally governed by a change in the molecular polarizability during a vibration; a mode is Raman-active only if the polarizability tensor changes along the normal coordinate. This selection rule is complementary to that of infrared absorption, which requires a change in dipole moment, and the two techniques are therefore often described as complementary rather than redundant (Larkin, 2017).

2.2 Instrumentation

A conventional dispersive Raman spectrometer consists of a monochromatic excitation source (typically a diode, argon-ion, or Nd:YAG laser operating between 405 and 1064 nm), a set of notch or edge filters to reject the intense Rayleigh line, collection optics to gather the weak scattered signal, a spectrograph to disperse the light by wavelength, and a charge-coupled device (CCD) detector (Larkin, 2017). Confocal microscope-coupled Raman systems additionally permit spatially resolved (micro-Raman) measurements with sub-micrometre lateral resolution, which has been central to applications in semiconductor defect mapping and two-dimensional material characterization (Ni et al., 2008).

Figure 2. Schematic layout of a dispersive Raman spectrometer

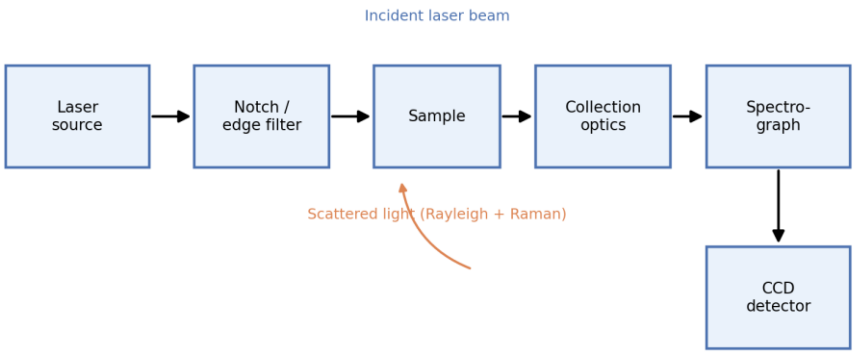

Figure 2. Schematic layout of a dispersive Raman spectrometer, showing the excitation, filtering, dispersion, and detection stages.

2.3 Practical Considerations and Limitations

Several practical factors govern the quality of a Raman measurement. Fluorescence from the sample or from trace impurities can generate a broad background that overwhelms the comparatively weak Raman signal; this is often mitigated by shifting to longer excitation wavelengths (e.g., 785 or 1064 nm), by photobleaching the sample prior to acquisition, or by applying baseline-correction algorithms such as asymmetric least squares during data processing (Zhang et al., 2025). Laser-induced heating and photodegradation are additional concerns for thermally or photochemically sensitive samples, including many biological and polymeric materials. They are usually controlled by limiting incident power density and exposure time. Because the Raman cross-section is intrinsically small, signal averaging over multiple accumulations, or the enhancement strategies described in Section 3, is often required to achieve an adequate signal-to-noise ratio, particularly for dilute analytes or short acquisition times demanded by imaging applications.

3. Enhanced and Advanced Raman Techniques

Because spontaneous Raman scattering is intrinsically weak, several enhancement and nonlinear strategies have been developed to improve sensitivity, spatial resolution, or acquisition speed.

3.1 Surface-Enhanced Raman Spectroscopy (SERS)

SERS exploits the electromagnetic field enhancement generated at the surface of nanostructured noble-metal substrates (typically gold or silver) through localized surface plasmon resonance, together with a smaller chemical enhancement contribution arising from charge transfer between the analyte and the metal surface. Enhancement factors of 10^4 to 10^8, and in some "hot-spot" geometries up to 10^{10}, allow single-molecule-level detection sensitivity (Le Ru & Etchegoin, 2008). Two-dimensional materials such as graphene and transition-metal dichalcogenides have more recently been explored as SERS substrates or hybrid metal/2D-material platforms, offering more reproducible and chemically tunable enhancement than purely metallic nanostructures (Xia, 2017).

3.2 Resonance Raman, Tip-Enhanced, and Coherent Raman Scattering

Resonance Raman spectroscopy is obtained when the excitation wavelength coincides with an electronic absorption band of the analyte, selectively enhancing the Raman bands of vibrational modes coupled to that electronic transition by several orders of magnitude; this selectivity is particularly valuable for probing chromophores in metalloproteins and pigments. Tip-enhanced Raman spectroscopy (TERS) combines the field enhancement of a scanning-probe metallic tip with the spatial resolution of scanning probe microscopy, achieving nanometre-scale chemical imaging beyond the optical diffraction limit. Coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS) are two examples of nonlinear coherent techniques that employ two or more synchronized laser beams to induce a coherent vibrational response, generating coherent signals that can be hundreds of orders of magnitude larger than the spontaneous Raman scattering and thus serve as the basis for video-rate label-free microscopy of living cells and tissues (Ranasinghe et al., 2023).

Table 1- *Comparison of principal Raman spectroscopic techniques*

Technique	Enhancement mechanism	Typical enhancement factor	Representative source
Spontaneous Raman	None (inherent polarizability change)	Baseline (1×)	Larkin (2017)

Technique	Enhancement mechanism	Typical enhancement factor	Representative source
Resonance Raman	Electronic resonance with chromophore	10^2–10^6	Larkin (2017)
SERS	Localized surface plasmon resonance + charge transfer	10^4–10^8 (up to 10^{10} at hot spots)	Le Ru & Etchegoin (2008)
TERS	Plasmonic tip field confinement	10^4–10^6 (nanoscale resolution)	Kudelski (2019)
CARS / SRS	Coherent nonlinear four-wave mixing	10^3–10^5 (signal strength)	Ranasinghe et al. (2023)

4. Applications of Raman Spectroscopy

Raman spectroscopy and imaging are used to determine the number and stacking order of graphene layers through the characteristic G and 2D (G′) bands, whose position, width, and relative intensity change systematically with layer number (Ni et al., 2008). For the micro- and nano-machining, Raman spectroscopy is used to measure the defects in crystal, phase changes and residual stress in semiconductor materials by the shift and broadening of the phonon characteristic bands (Zhu et al., 2018). In biomedicine, Raman-based methods, including SERS, have found applications as rapid imaging methods that complement conventional techniques like MRI and PET for the detection of cancer, cardiovascular disease, neurodegenerative disorders and viral infection biomarkers in blood, saliva and tissue (Ranasinghe et al., 2023; Yang et al., 2023). Raman spectroscopy has additionally found use in non-invasive assessment of gametes and embryos in assisted reproduction (Karimi et al., 2025) and in operando catalytic studies that track the vibrational signatures of reaction intermediates on catalyst surfaces under working conditions.

Table 2- *Representative Raman shift ranges and associated structural information*

Raman shift range (cm^{-1})	Vibrational assignment	Typical application
100–500	Lattice / phonon modes, metal–ligand stretches	Crystal phase identification; inorganic and mineral analysis
500–800	C–S, C–Cl, ring deformation	Polymer and halogenated compound identification
1000–1100	C–C, C–O, C–N skeletal stretches	Carbohydrate and nucleic acid backbone analysis
1300–1400	D band (disordered carbon), CH bending	Carbon-material defect density (graphene, CNTs)
1580–1600	G band (sp^2 carbon), aromatic ring stretch	Graphitic/graphene layer number and quality
2500–2700	2D (G′) overtone band	Graphene layer-number determination

Raman shift range (cm^{-1})	Vibrational assignment	Typical application
2800–3100	C–H, N–H, O–H stretches	Lipid, protein, and general organic fingerprinting

Note. Ranges compiled from Larkin (2017) and Ni et al. (2008).

Beyond the specific bands summarized in Table 2, Raman imaging extends single-point spectroscopy into two- and three-dimensional chemical mapping. The key features of Raman imaging involve scanning a focused laser spot across a sample and capturing a full spectrum at each laser-illuminated spot to obtain maps of composition, crystallinity, or stress that can be very useful for locating localized defects on a machined semiconductor surface, for visualizing the drug distribution within a pharmaceutical tablet, or in the identification of the healthy and diseased tissue margins within an ex vivo biomedical sample (Zhu et al., 2018; Ranasinghe et al., 2023). When combined with multivariate chemometric techniques, such as principal component analysis and, more recently, deep neural networks, automated classification of spectra based on the visual inspection becomes more possible (Zhang et al., 2025).

In addition to materials and biomedical science, Raman spectroscopy has found many applications in art conservation and cultural heritage studies, for example, identification of paint components, pigments and binders in paintings and manuscripts without removing any material, and in food science for the detection of food adulteration, for the authentication of food and for on-line monitoring of food fermentation and ripening processes. Portable Raman instruments are also employed in the field in environmental and forensic applications to identify unknown solids and liquids, including hazardous materials and controlled substances, because the technique is rapid and can be performed in transparent containers, like glass or plastic. These diverse use cases illustrate a common theme: because the Raman spectrum encodes chemical composition directly as a fingerprint, the technique translates readily across disciplines that share little else in common methodologically.

5. Fundamentals of NMR Spectroscopy

Nuclear magnetic resonance arises because nuclei with non-zero spin (such as ^{1}H, ^{13}C, ^{15}N, and ^{31}P) possess a magnetic moment that, when placed in a strong external magnetic field, precesses at a characteristic Larmor frequency proportional to the field strength and the nucleus's gyromagnetic ratio. Irradiation with a radiofrequency pulse at or near this frequency tips the bulk nuclear magnetization away from equilibrium; the subsequent free induction decay, Fourier-transformed into the frequency domain, yields the familiar NMR spectrum, in which each chemically distinct nucleus resonates at a characteristic chemical shift determined by its local electronic environment (Claridge, 2016).

One-dimensional ^{1}H and ^{13}C NMR spectra provide chemical shift, integration, and scalar (J) coupling information, but resonance overlap becomes severe as molecular size and complexity increase. This limitation, together with the desire to measure through-bond and through-space connectivities directly, motivated the development of multi-dimensional NMR, in which magnetization is allowed to evolve during one or more incremented delay periods before detection, spreading the resulting correlations across two or more frequency axes (Claridge, 2016; Wüthrich, 1986).

5.1 NMR Instrumentation

Modern high-resolution NMR spectrometers are built around superconducting magnets, typically operating between 400 and 1000 MHz ^{1}H resonance frequency, cooled with liquid helium to maintain the persistent current that generates the static field. Samples are held in narrow-bore glass tubes positioned within a radiofrequency probe that both transmits the excitation pulses and detects the resulting signal; cryogenically

cooled probes reduce electronic noise and can improve sensitivity several-fold relative to room-temperature probes, which is particularly valuable for mass-limited biological samples. Pulsed field gradients, incorporated into most modern probes, are used to select coherence pathways and suppress unwanted signals such as residual solvent resonances, substantially shortening experiment times relative to the phase-cycling schemes used in earlier instrument generations (Claridge, 2016). For biomolecular applications, samples are commonly enriched with ^{13}C and ^{15}N, and in some cases partially or fully deuterated, to enable the heteronuclear and triple-resonance experiments described in Section 6.

Table 3- *Typical ^{1}H and ^{13}C chemical shift ranges used in spectral assignment*

Functional group	^{1}H shift (ppm)	Functional group (^{13}C)	^{13}C shift (ppm)
Alkyl CH_3 / CH_2	0.7–1.5	Alkyl C	10–45
CH adjacent to C=O	2.0–2.6	C adjacent to C=O	30–45
O–CH_3 / O–CH_2	3.3–4.5	C–O (aliphatic)	55–80
Vinylic / aromatic CH	5.0–8.5	Aromatic / vinylic C	100–150
Aldehyde CH	9.5–10.0	Carbonyl C (ester/amide)	160–175
Carboxylic acid OH	10–12 (broad)	Carbonyl C (ketone/aldehyde)	190–210

Note. Ranges compiled from Claridge (2016); values are approximate and solvent-dependent.

6. Multi-Dimensional NMR Techniques

Multi-dimensional NMR experiments are broadly classified as homonuclear, correlating nuclei of the same isotope (typically ^{1}H), or heteronuclear, correlating ^{1}H with a second nucleus such as ^{13}C or ^{15}N. A two-dimensional experiment consists of a preparation period, an incremented evolution period (t_1), a mixing period during which magnetization is transferred between coupled spins, and a detection period (t_2); Fourier transformation with respect to both time domains produces a two-dimensional spectrum with diagonal peaks (corresponding to the conventional one-dimensional spectrum) and off-diagonal cross peaks that reveal coupling or spatial proximity between nuclei (Claridge, 2016).

6.1 Correlation Spectroscopy (COSY) and Total Correlation Spectroscopy (TOCSY)

COSY correlates protons that are scalar-coupled through two or three bonds, producing cross peaks that map out the connectivity of a spin system and are essential for assigning proton resonances in organic molecules such as alkaloids and steroids (Advances in Polymer Science, n.d.). TOCSY extends this correlation across an entire spin system via a relayed magnetization-transfer sequence, which is particularly useful for identifying interconnected spin networks of individual sugar or amino-acid residues in oligosaccharides, peptides, and metabolomic mixtures.

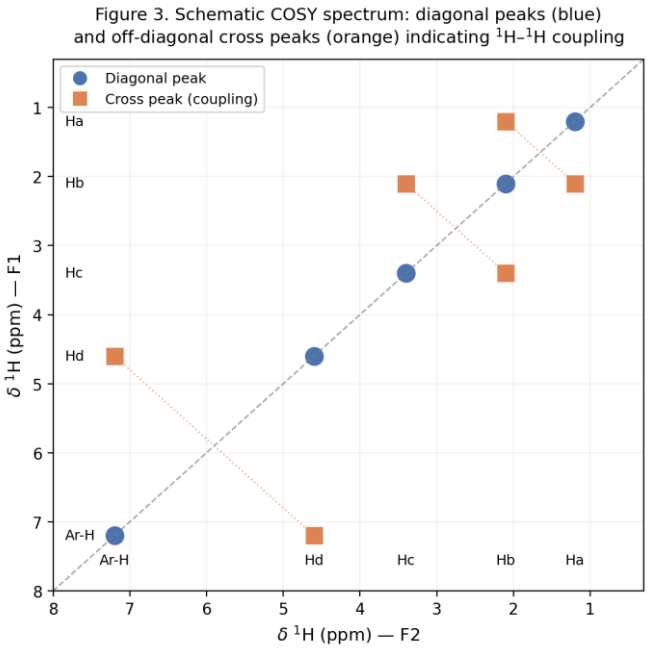

Figure 3. Schematic COSY spectrum illustrating diagonal peaks and symmetric off-diagonal cross peaks that reveal scalar-coupled proton pairs.

6.2 Nuclear Overhauser Effect Spectroscopy (NOESY)

NOESY detects cross-relaxation (the nuclear Overhauser effect) between protons that are close in space, typically within about 5 Å, irrespective of whether they are scalar-coupled. Because NOESY cross-peak intensities can be converted into approximate inter-proton distances, the technique is central to determining the three-dimensional solution structure of peptides, oligonucleotides, and small proteins, and is used together with sequence-specific resonance assignments to identify secondary-structure elements such as helices, sheets, and loops (News-Medical, 2019).

6.3 Heteronuclear Single-Quantum Coherence (HSQC) and Heteronuclear Multiple-Bond Correlation (HMBC)

HSQC correlates each proton with the heteronucleus (typically ^{13}C or ^{15}N) to which it is directly bonded, producing one cross peak per C–H or N–H pair and dramatically improving spectral resolution relative to homonuclear methods (Advances in Polymer Science, n.d.). The methyl region of the ^{1}H–^{13}C HSQC spectrum has, for example, been shown to be sensitive to both secondary and tertiary protein structure, making it a valuable tool for assessing the higher-order structural integrity of therapeutic proteins (Arbogast et al., 2021). HMBC, by contrast, detects longer-range (typically two- to four-bond) ^{1}H–^{13}C correlations, allowing connectivity to be established across quaternary carbons and heteroatoms that HSQC cannot directly probe, which is essential for assembling complete carbon skeletons of complex natural products.

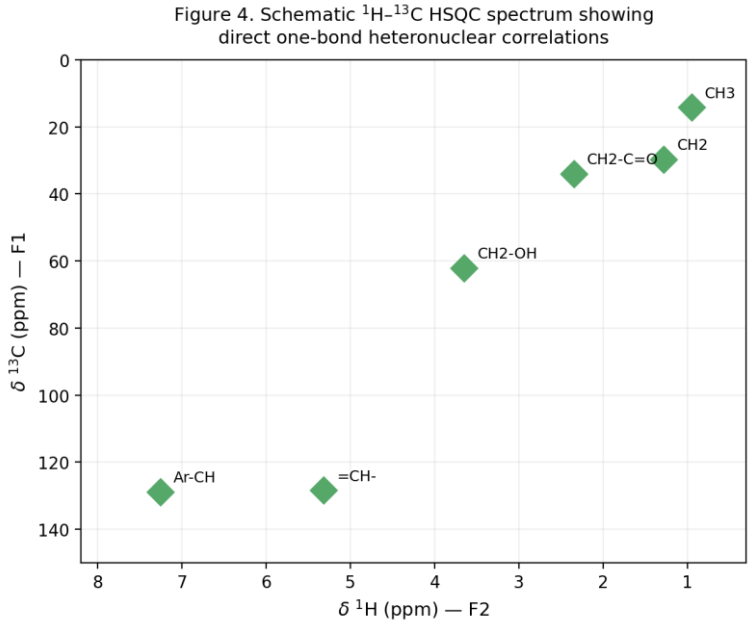

Figure 4. Schematic ¹H–¹³C HSQC spectrum showing direct one-bond heteronuclear correlations for representative functional groups.

6.4 Three- and Four-Dimensional NMR

For large biomolecules, even two-dimensional spectra suffer from severe resonance overlap. Three-dimensional experiments, such as ^{15}N-edited TOCSY-HSQC and NOESY-HSQC, and triple-resonance experiments such as HNCA and HNCO, resolve this overlap by adding a third heteronuclear frequency axis, enabling sequential backbone assignment of proteins several hundred residues in length (Deng et al., 2011; Ranasinghe et al., 2023 conceptual analogue in Raman literature notwithstanding, the analogous NMR development is documented in Deng et al., 2011). Four-dimensional experiments, such as the NOE–NOE experiment used in the study of the SARS-CoV-2 main protease, extend this strategy further, and combined with perdeuteration and transverse relaxation-optimized spectroscopy (TROSY), have enabled structural analysis of proteins as large as several hundred kilodaltons (Tugarinov et al., 2002; Xiao et al., 2023).

Table 4- *Summary of principal multi-dimensional NMR experiments*

Experiment	Correlation type	Information obtained	Representative source
COSY	^{1}H–^{1}H (2–3 bond, scalar)	Vicinal/geminal proton connectivity	Advances in Polymer Science (n.d.)
TOCSY	^{1}H–^{1}H (relayed, whole spin system)	Spin-system networks (sugars, residues)	Advances in Polymer Science (n.d.)
NOESY	^{1}H–^{1}H (through-space, ≤5 Å)	Inter-proton distances; 3D structure	News-Medical (2019)
HSQC	^{1}H–^{13}C / ^{1}H–^{15}N (1-bond)	Direct C–H / N–H connectivity	Arbogast et al. (2021)
HMBC	^{1}H–^{13}C (2–4 bond)	Long-range skeletal connectivity	Advances in Polymer Science (n.d.)

Experiment	Correlation type	Information obtained	Representative source
3D HNCA / HNCO	$^{1}H–^{15}N–^{13}C$ (triple resonance)	Sequential protein backbone assignment	Deng et al. (2011)
4D NOE–NOE	$^{1}H–^{1}H$ (double heteronuclear-edited)	Assignment and structure in large proteins	Xiao et al. (2023)

7. Applications of Multi-Dimensional NMR

In addition to 3D heteronuclear experiments, sequential combinations of 2D and 3D heteronuclear experiments are still the main solution-state method for structure determination of protein and nucleic acid structures, complementing X-ray crystallography and cryo-EM, and also provide information about conformational dynamics in solution (Wüthrich, 1986; Xiao et al., 2023). 2D $^{1}H–^{13}C$ HSQC has been embraced for its sensitivity to subtle conformational changes by lower-resolution biophysical methods like circular dichroism, which makes it a higher-order structure fingerprinting method for pharmaceutical and biopharmaceutical analysis, and specifically, for the analysis of monoclonal antibodies (Arbogast et al., 2021). TOCSY and HSQC are two-dimensional NMR experiments that are used to separate overlapping resonances in complex biological samples like blood serum and urine to allow dozens of metabolites to be identified simultaneously without any other purification step. In materials science, HSQC and related heteronuclear experiments are used to determine repeat-unit structure and branching patterns in biopolymers such as cellulose and chitosan, as well as in synthetic polymers and composites (Advances in Polymer Science, n.d.). Multi-dimensional NMR also underpins quantitative studies of molecular dynamics. Relaxation-dispersion and Carr–Purcell–Meiboom–Gill (CPMG) experiments, layered onto standard heteronuclear correlation sequences, report on conformational exchange processes occurring on microsecond-to-millisecond timescales, information that is largely inaccessible to static structural methods such as X-ray crystallography. In natural product chemistry, the combined use of COSY, HSQC, and HMBC spectra remains the standard workflow for de novo structure elucidation of newly isolated compounds, allowing complete carbon and proton assignment, including quaternary centres, without reliance on comparison to a reference database (Claridge, 2016).

Multi-dimensional NMR is also central to pharmaceutical metabolite identification, where HSQC and HMBC spectra of isolated drug metabolites establish the position of biotransformation on the parent molecule, information that is often difficult to obtain unambiguously from mass spectrometry alone. In food and beverage authentication, 2D NMR fingerprints of complex mixtures such as wine, honey, or olive oil provide a quantitative, reproducible basis for detecting adulteration or verifying geographic origin, complementing the more rapid but less specific screening that Raman spectroscopy can provide for the same samples.

8. Comparative Analysis: Raman Spectroscopy and Multi-Dimensional NMR

Raman spectroscopy and multi-dimensional NMR are frequently described as complementary rather than competing techniques. Raman spectroscopy requires minimal sample preparation, is compatible with aqueous and solid samples alike, provides rapid (often sub-second to minute-scale) acquisition, and can be performed with portable or microscope-coupled instrumentation suitable for in situ and in vivo measurements. However, spontaneous Raman signals are weak, fluorescence background can obscure spectral features, and the technique provides comparatively coarse information about three-dimensional molecular conformation. Multi-dimensional NMR, by contrast, offers atomic-resolution structural and dynamic information, including precise inter-nuclear distances and torsion angles, but requires more concentrated samples, longer acquisition

times (from minutes for small molecules to days for large proteins), and access to high-field superconducting magnets that represent a substantial capital investment (Claridge, 2016; Larkin, 2017).

Table 5- *Comparative characteristics of Raman spectroscopy and multi-dimensional NMR*

Characteristic	Raman spectroscopy	Multi-dimensional NMR
Typical sample state	Solid, liquid, gas; minimal preparation	Solution (or solid-state with specialized probes)
Typical acquisition time	Seconds to minutes	Minutes (small molecules) to days (proteins)
Structural resolution	Functional-group / vibrational fingerprint	Atomic-resolution 3D structure and dynamics
Sensitivity limitation	Weak spontaneous signal; fluorescence interference	Low intrinsic sensitivity of nuclear spins
Instrumentation cost/footprint	Low to moderate; benchtop and portable options	High; requires superconducting magnet
Representative strength	Rapid fingerprinting, imaging, in situ monitoring	Definitive structure elucidation and dynamics

In practice, the two techniques are not necessarily used in turn, but Raman spectroscopy can give a fast-initial screening of the chemical composition or crystallinity, while multi-dimensional NMR can be used for definitive structure determination on a prioritized specimen. In the pharmaceutical industry, for example, Raman imaging is routinely applied to high-throughput polymorph screening of drug formulations and NMR to the atomic-level identity and purity of a selected polymorph candidate. Similarly, in structural biology, Raman spectroscopy can determine the secondary structure of a protein in solution in a short time, and multi-dimensional NMR (or a combination of NMR with X-ray crystallography and cryo-electron microscopy) can provide the definitive model of the protein at atomic resolution (Wüthrich, 1986; Xiao et al., 2023).

9. New Directions and Emerging Trends

The fields are undergoing transformations with the advancement of data analysis and instrumentation. Raman deep-learning techniques are now being used to preprocess the spectra, correct for baseline, classify, and quantify (Zhang et al., 2025) instead of manual chemometric modeling, making them more robust to noisy, heterogenous data. The use of hyphenated and multimodal approaches, such as Raman imaging and machine-learning-based classification, are being investigated for real-time clinical diagnostics of brain tumors and neurodegenerative diseases (Ranasinghe et al., 2023). Historically, portable/handheld Raman instruments were used for qualitative screening, but now they can perform quantitative analysis of materials in the field – from pharmaceutical anti-counterfeiting to on-site mineral identification – and cut the time between sample collection and result when compared to sending materials to a central laboratory.

There are still larger proteins that can be solved with solution state NMR using this combination of cryoprobes, non-uniform sampling and higher magnetic field, and techniques like TROSY and selective isotope labeling are pushing the size limit of protein complexes amenable to multi-dimensional NMR to megadalton scale (Xiao et al., 2023). In particular, non-uniform sampling schemes enable a substantial reduction in the duration of experiments by orders of magnitude by only sampling the indirect time domain dimensions of 3D and 4D

experiments, while retaining a comparable level of resolution in the calculation. The introduction of automated data processing pipelines (e.g., NMRPipe, CcpNmr) into the pipeline of NMR structure determination has made the process much easier to use by alleviating the manual assignment burden that was previously prominent in the field (Bhattacharya et al., 2011). More advanced are the dynamic nuclear polarization and para-hydrogen-based hyperpolarization techniques that could enhance the sensitivity of NMR in the future by several orders of magnitude, reducing the sensitivity difference with optical spectroscopies like Raman.

10. Conclusion

Raman scattering and multi-dimensional NMR spectroscopy, though grounded in distinct physical phenomena, together provide a remarkably comprehensive toolkit for probing molecular structure, composition, and dynamics. Raman spectroscopy, enhanced through surface-, tip-, and resonance-based strategies, offers rapid, minimally invasive fingerprinting well suited to materials characterization, nanomaterial metrology, and emerging biomedical diagnostics. Multi-dimensional NMR, spanning homonuclear COSY and NOESY through heteronuclear HSQC, HMBC, and higher-dimensional triple-resonance experiments, remains the definitive solution-state method for atomic-resolution structure determination of organic molecules, biopolymers, and proteins.

The comparative analysis presented in this review underscores that the choice between these techniques, or the decision to combine them, should be guided by the specific structural question at hand: rapid fingerprinting and imaging favor Raman spectroscopy, whereas definitive three-dimensional structure and dynamics call for multi-dimensional NMR. As instrumentation, isotope-labeling strategies, and computational tools continue to mature, particularly through the integration of machine learning and hyperpolarization methods, the combined and complementary use of Raman and multi-dimensional NMR spectroscopy is likely to expand further into in situ, in vivo, and high-throughput structural analysis. Researchers designing new structural studies would therefore do well to treat these two spectroscopic families not as alternatives but as sequential or parallel stages of a single, more powerful analytical strategy, reinforcing their status as foundational techniques of the modern spectroscopic sciences.

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