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Raman spectroscopic characterisation of leaves for plant species discrimination

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Abstract

Spectroscopic techniques are rapidly gaining traction in remote sensing due to their ability to detect subtle biochemical and structural variations in vegetation. Raman spectroscopy, in particular, provides molecular-level insight into plant tissues by capturing vibrational modes of constituent biomolecules. This study harnessed the power of a 532 nm excitation source to meticulously obtain Raman spectra from two distinct leaf samples, with a keen focus on uncovering their spectral differences that reflect the intricate biomolecular composition of green plant tissues. The spectral profiles revealed three prominent peaks situated around 1008 cm^{-1}, 1156 cm^{-1}, and 1520 cm^{-1}, each corresponding to the vibrational modes of carotenoid molecules that play a crucial role in photosynthesis. Notably, Leaf 1 exhibited significantly higher peak intensities compared to Leaf 2, suggesting an elevated concentration of carotenoids and, implicitly, a greater photosynthetic activity. These discernible spectral differences are not merely academic; they are crucial for identifying diverse forest species and monitoring crops. Raman spectroscopy, with its capacity for swift, in situ biochemical fingerprinting, contributes to the development of comprehensive spectral libraries. Such libraries are invaluable for species classification in ecologically rich environments. In agricultural contexts, this cutting-edge technique serves as an early warning system, enabling the monitoring of crop health and the detection of nutrient deficiencies long before visible symptoms appear, thereby enhancing precision agriculture practices. From a geospatial perspective, Raman spectroscopy emerges as a critical ground-truthing tool. It provides essential molecular data used to calibrate satellite-derived vegetation indices, which in turn bolsters the accuracy of machine learning models. When integrated with Geographic Information Systems (GIS) platforms, field-portable Raman devices can generate detailed biochemical maps that facilitate multi-scale vegetation monitoring, leveraging the strengths of UAVs and satellite sensors. The choice of a 532 nm laser is pivotal, as it significantly amplifies sensitivity to carotenoids, making Raman spectroscopy exceptionally well-suited for nuanced studies of green vegetation, whether in pristine natural ecosystems or cultivated managed landscapes. Overall, this innovative technique holds immense promise for advancing vegetation monitoring efforts and supporting informed, climate-smart decision-making in environmental and agricultural management.

1. Introduction

Spectroscopic techniques are gaining importance in the field of remote sensing due to their capability to detect subtle molecular and structural differences in natural materials. Among these techniques, Raman spectroscopy stands out for its advantages as a non-invasive, label-free method that provides high-resolution insights into the vibrational modes of specific molecular bonds. This unique ability to identify and differentiate biochemical components at the molecular level makes Raman spectroscopy an invaluable tool for analyzing complex biological, geological, and environmental systems. Its application has been particularly extensive in the physical sciences, where it has been utilized to investigate ice structures and various mineralogical phases.

Raman spectra of ice Ih offer a fascinating glimpse into the intricate dynamics of hydrogen bonds. Notably, the most prominent band appears at 3150 cm^{-1} within the cc polarization component, which elegantly captures the unique structural configuration of ice. This spectral feature not only highlights the strength and arrangement of the hydrogen bonds but also enhances our understanding of the complex interactions that define the properties of ice (Wang et al., 2019). The spectral features we observe provide valuable insights into how temperature variations can affect hydrogen bonding networks. This underscores the technique's effectiveness in monitoring and analyzing environmental changes, allowing for a deeper understanding of the underlying processes at play. Further research has verified that Raman spectroscopy consistently identifies the vibrational modes of water molecules, such as symmetric and antisymmetric OH stretching and HOH bending. These spectral characteristics are influenced by structural changes or external conditions like temperature and pressure (Rull et al., 2011). Recent advancements in Raman spectroscopy technology, coupled with improvements in data processing and the integration of machine learning, have broadened its applications beyond traditional laboratory-based material analysis. This evolution allows for more versatile and efficient analysis in various fields, enhancing the capability to explore materials in real-world settings. Remote Raman spectroscopy has been explored for environmental and aquatic sensing, such as the detection of dissolved oxygen and nitrogen in coastal waters from airborne platforms (Ganoe & DeYoung, 2013). These capabilities demonstrate the potential of Raman systems for real-time, large-scale environmental monitoring. Similarly, Raman spectroscopy combined with chemometric methods has enabled precise classification of soil samples, significantly reducing laboratory costs and enhancing field-based analytical throughput (Luna et al., 2014). In planetary science, machine learning techniques such as convolutional neural networks have been successfully applied to Raman spectra for mineral identification under conditions simulating the Martian surface. The high classification accuracy achieved using deep learning on low-signal spectra underscores the robustness and adaptability of Raman-based methods for geospatial exploration and autonomous planetary missions (Berlanga et al., 2022). Furthermore, the ability of Raman spectroscopy to distinguish mineral polymorphs, such as calcite and aragonite, based on their lattice vibrations, provides an advantage over other spectroscopic techniques that cannot resolve polymorphic variations (Pasteris & Beyssac, 2020). Despite these technological advancements, the direct comparative application of Raman spectroscopy for analyzing and distinguishing different plant species based on their pigment-related vibrational characteristics remains underexplored. Specifically, limited studies have investigated the variation in carotenoid-associated Raman peaks across distinct

vegetation types, especially within forest tree species and broadleaf ornamental plants. The gap in spectral libraries for such species constrains the utility of Raman spectroscopy in ecosystem characterization and spectral unmixing applications. The motivation for the present study arises from the need to expand the Raman spectral knowledge base for plant pigments and to assess its capability for differentiating vegetation types based on biochemical signatures. Carotenoids, being essential components of the photosynthetic apparatus, exhibit strong resonance with 532 nm laser excitation, making this wavelength particularly suited for Raman spectroscopic studies of green vegetation. The capacity to detect variations in carotenoid content offers an opportunity to link Raman spectral data with physiological traits relevant to both forestry and agriculture.
The objective of this study is to analyse and interpret the Raman spectra obtained from Agathis robusta and Syngonium podophyllum leaves, with a focus on carotenoid-associated vibrational bands and their relative intensities. The study further seeks to demonstrate how these Raman spectral features can support geospatial vegetation classification, enhance spectral library development for vegetation, and contribute to remote sensing applications in forest species identification and agricultural crop monitoring.

2. Importance of Agathis robusta (Queensland kauri) and Syngonium podophyllum (arrowhead plant)

Agathis robusta, commonly known as the Queensland kauri, and Syngonium podophyllum, often referred to as the arrowhead plant, exemplify two remarkably distinct taxa in the plant kingdom. The Queensland kauri is an ancient coniferous gymnosperm, characterized by its towering stature and impressive longevity, thriving in the lush forests of northeastern Australia. In contrast, the arrowhead plant is a widely distributed angiosperm belonging to the aroid family, known for its distinctive, arrow-shaped leaves and ability to adapt to a variety of environmental conditions. Each species occupies its own unique ecological niche, contributing to biodiversity and ecosystem stability. Furthermore, they hold significant biochemical properties and socio-economic value, with the Queensland kauri's timber prized for its durability and aesthetic appeal, while Syngonium podophyllum is favored in ornamental horticulture and has several traditional medicinal uses. A critical analysis of these species, augmented by recent advances in Raman spectroscopy, yields comprehensive insights that span molecular chemistry, ecosystem services, and community relevance. Agathis robusta is indigenous to Australia and Papua New Guinea, forming canopy emergents in rainforests and well-drained, humid to subhumid soils. Robust both in girth and longevity, Agathis robusta can reach over 40 meters, contributing to the architecture and succession patterns of their native forests (Whitmore, 1980). The tree associates with diverse fauna; for instance, its seeds serve as food for cockatoos, while its foliage hosts an array of Lepidopteran larvae. As a slow-growing and unaggressive species, it is not considered invasive in global forestry, yet it supplies substantial ecosystem services by stabilizing soils and supporting biodiversity (Barwick, Van der Schans, & Barwick Claudy, 2004). From a socio-economic viewpoint, Agathis robusta has historically been valued for its premium, knot-free timber, employed in boat-building, furniture, and fine joinery. Its oleoresin has found traditional use and its timber continues to be a desirable forest product, although current conservation regulations are increasingly important as wild populations are threatened by localized logging in Papua New Guinea.

Phytochemical investigations reveal Agathis robusta as a source of glycosides, flavonoids, saponins, and tannins (Ahmed & Mohamed, 2022). The aerial parts of the plant, when extracted with methanol, yield both triterpene saponins and flavonoids that demonstrate promising hepatoprotective activities in both in vitro and in vivo studies. Remarkably, these activities are comparable to or even exceed those of conventional reference drugs like silymarin. Furthermore, research has identified two novel triterpenoidal saponins using two-dimensional NMR and mass spectrometric techniques. These saponins have shown encouraging dose-dependent reductions in serum markers of liver damage (ALT and AST) in Wistar rats exposed to CCl_4 (Ahmed & Mohamed, 2022). This highlights the potential therapeutic benefits of these compounds in liver health management. Complementary studies have expanded the profile of Agathis robusta bark, identifying over 95 metabolites, principally biflavonoids, diterpenoid acids, and phenolic acids, which display promising molecular interactions with inflammation and cell death mediators (Mohamed, Tawfeek, Elbaramawi, Elbatreek, & Fikry, 2022). These findings align with prior evidence that essential oils and extracts from Agathis robusta possess antibacterial and anti-inflammatory properties, extending their pharmaceutical and nutraceutical potential.

Syngonium podophyllum, native to moist Central and South American forests, is an ornamental climber prized in horticulture for its foliage and tolerance to a range of indoor conditions. Syngonium podophyllum is notable for its ecological significance, particularly in the context of phytoremediation. This species is effective at absorbing volatile organic compounds such as benzene, toluene, and formaldehyde, making it a natural air purifier for indoor environments (Balan & Chandrasekaran, 2022). It can thrive in various settings, which not only enhances local environmental quality but also contributes to household well-being due to its ease of cultivation.

Biochemically, Syngonium podophyllum stands out due to its relatively high concentrations of phenolic compounds and moderate levels of flavonoids, as revealed by aqueous leaf extracts (Kumar, Kumar, Dwivedi, & Pandey, 2014). This intriguing plant is enriched with a diverse array of secondary metabolites, including flavonoids, terpenoids, alkaloids, and saponins, each contributing to its wide-ranging biological activities. While the antioxidant properties of its leaf extracts may not rival those of more potent aquatic plants, they are still significant, as evidenced by various assays that measure reducing power, DPPH radical scavenging capability, and the inhibition of lipid peroxidation.

Moreover, these aqueous extracts exhibit noteworthy antibacterial effects, particularly against Gram-negative bacteria such as Proteus vulgaris and Salmonella typhi, indicating their potential as natural antimicrobial agents. Additionally, laboratory studies demonstrate that the extracts have cytotoxic effects against several human cancer cell lines in vitro (Kumar et al., 2014). However, it is essential to exercise caution, as extracts from Syngonium podophyllum have also been linked to hepatotoxic effects in animal models, underscoring the importance of careful consideration when contemplating its medicinal applications.

Socio-economically, Syngonium podophyllum's principal value resides in its horticultural appeal and air-purifying efficacy. Endorsed by several studies and widely adopted in urban environments, the species contributes to healthier living spaces and participates in green, sustainable remediation strategies. Its low maintenance requirements foster both commercial trade and individual well-being through accessible greening of indoor environments. A significant trend in the research surrounding Agathis robusta and Syngonium podophyllum is the innovative use of Raman spectroscopy. This advanced vibrational spectroscopic technique facilitates a non-invasive, label-free approach to rapidly fingerprint the intricate biochemistry of these plants. In the field of plant science, Raman spectroscopy provides valuable insights by enabling the quantification and detailed mapping of secondary metabolites (Payne & Kurouski, 2021). These compounds are not only crucial to the pharmaceutical properties of these species but also play vital roles in their ecological interactions. Raman peaks associated with lignin or phenolic structures can distinguish plant tissues, guide breeding for disease resistance, or gauge responses to environmental stressors (Jain, Rose, Jayapal, Singh, & Ram, 2024; Weng et al., 2021).

The versatility of Raman spectroscopy extends to real-time, in situ ecosystem assessments—enabling researchers and practitioners to monitor plant stress, nutrient status, or contamination. A plant's unique Raman spectrum can be used for species identification, physiological status assessment, and to distinguish between healthy and stressed individuals (Jain et al., 2024; Payne & Kurouski, 2021). Although specific published work on Raman spectroscopy in Agathis robusta and Syngonium podophyllum remains nascent, the documented Raman signatures of plant secondary chemistry pave the way for targeted studies that could, for instance, track saponin, flavonoid, or lignin content throughout ecological interactions or during phytoremediation events. Zavafer & Ball, 2023 reviewed the intricacies of Raman band assignments for various components in plant material, including pigments, structural and non-structural carbohydrates, lipids, proteins, and secondary metabolites (Zavafer & Ball, 2023). This analysis highlights the exciting opportunities that this advanced technology presents for deepening our understanding of vascular plant physiology and the complex biochemical interactions within these organisms. As Raman technology advances and datasets grow, Raman-based assessments promise to accelerate basic research, commercial quality control, conservation monitoring, and the sustainable management of both iconic conifers like Agathis robusta and versatile ornamentals such as Syngonium podophyllum.

In sum, Agathis robusta and Syngonium podophyllum, through their distinct but complementary ecological and biochemical roles, demonstrate the intricate weave of nature, chemistry, and human systems. Agathis robusta, as a giant of ancient forests, supplies structural stability and valuable pharmaceuticals, while Syngonium podophyllum enhances urban life through air purification and ornamental culture. Raman spectroscopy now offers an unprecedented molecular window into these dynamics, facilitating a future where sustainable resource use, ecological monitoring, and molecular diagnostics coalesce for the benefit of both natural and human communities.

3. Methodology for Spectral Analysis using Raman Spectrometer

The methodology adopted to collect the Raman Spectra of Green leaves of Agathis robusta and Syngonium podophyllum is shown in Figure 1.
Raman spectra were meticulously collected using a high-resolution spectrometer, which was outfitted with a precise 532 nm excitation laser to ensure optimal data acquisition. Following the initial data collection, the raw spectral information underwent rigorous preprocessing to enhance the quality of the signals and facilitate accurate comparisons across samples. Once the spectra were preprocessed, detailed analysis was conducted to uncover significant vibrational features, with a particular focus on those associated with carotenoid-related molecular bonds. A comparative assessment was then performed on the spectral intensities and peak positions, enabling a thorough evaluation of the differences in pigment composition between the two species under investigation.

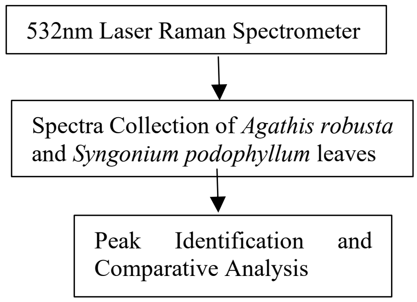

Figure 1. Methodological Flow Diagram

The resulting spectral signatures were interpreted in relation to the biochemical characteristics of the vegetation, providing valuable insights into their profiles. This analysis not only enhances our understanding of the plant species studied but also holds potential for advancing remote sensing and geospatial vegetation classification efforts, crucial for ecological monitoring and management.

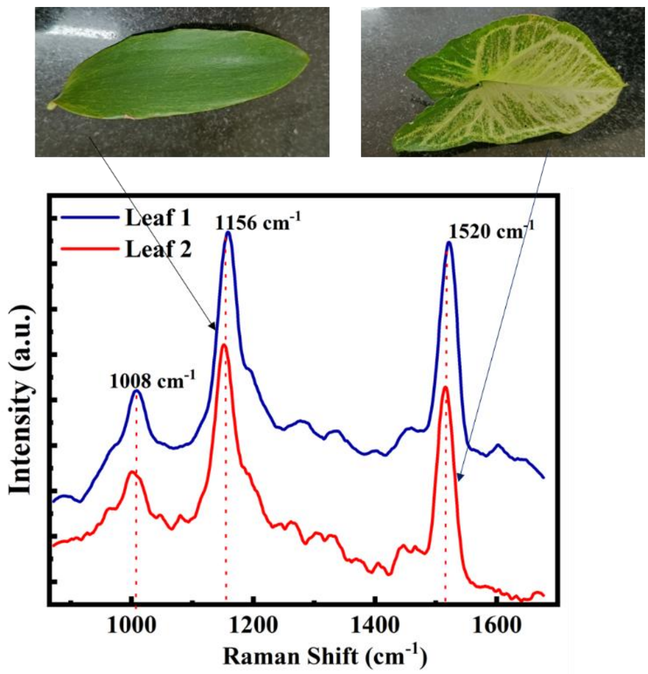

Figure 2: Spectral profile of *Agathis robusta* (Leaf 1) and *Syngonium podophyllum* (Leaf 2) leaves

The Raman spectrum shown for Agathis robusta (Leaf 1) and Syngonium podophyllum (Leaf 2) leaves, collected with a 532nm laser, offers a vivid molecular fingerprint revealing key aspects of their biochemical composition and structural specialization. In the spectral profile, both leaves display several prominent peaks, yet the differences in intensity, position, and sharpness of these features highlight their evolutionary and physiological distinctions. The most pronounced features in both spectra are the bands at approximately 1008cm^{-1}, 1156cm^{-1}, and 1520cm^{-1}. The 1008cm^{-1} band is commonly associated with C–O and C–C stretching vibrations in polysaccharides, especially cellulose and hemicellulose, compounds vital for plant cell wall integrity and mechanical strength. Agathis robusta, which shows a sharper and more intense peak at this position, likely possesses a higher abundance or crystallinity of such structural carbohydrates, reinforcing its robust, leathery leaf structure as a conifer adapted for longevity and mechanical durability. In contrast, Syngonium podophyllum's corresponding peak, though present, is less pronounced, consistent with its thin, flexible parenchymatous leaves that are typical of mesophytic angiosperms.

At 1156cm^{-1}, the strong band is a hallmark of C–O–C linkages in cellulosic and xylan backbones and again, Agathis robusta shows a significant intensity at this position. This suggests that coniferous foliage has evolved to feature more robust secondary wall constituents compared to the more herbaceous Syngonium, which displays a lower yet discernible signal at this wavenumber, likely reflecting the lower relative content or a different organization of wall biopolymers. These differences signify not only the taxonomic divergence but also the specific ecological adaptations of each species. The dominance of these polymer fingerprints in Agathis robusta's spectrum aligns with its ecological niche, long-lived, sun-exposed, and designed to withstand abiotic stress.

The 1520cm^{-1} peak seen in both spectra stems from aromatic ring stretching in phenolic compounds such as lignin and certain flavonoids. Agathis robusta again demonstrates a higher intensity, implying a greater investment in phenolic biochemistry, which supports not just mechanical reinforcement but also resistance against biotic stress and ultraviolet radiation. The aromatic region is often amplified in gymnosperms as a result of evolutionary pressures faced in exposed, competitive environments. In Syngonium podophyllum, although the 1520cm^{-1} peak is visible, its lower intensity points to softer tissue chemistry, with less lignification. This observation fits the biological reality of Syngonium's shade-adapted, rapidly growing, and climbing habit, which reduces the selective pressure for high lignin deposition.

The relative broadness and attenuation of Syngonium podophyllum's peaks compared to the sharper, stronger signals of Agathis robusta may also be indicative of differences in the molecular order or hydration states of tissue, and possibly greater metabolic plasticity. The chosen 532nm laser wavelength is particularly effective for resonantly enhancing the Raman response of pigments and aromatic compounds, thus offering useful insights into the distribution of biochemically active substances in both species (Payne et al., 2021; Jain et al., 2024).

These spectral differences, clearly depicted in the data, highlight how Raman spectroscopy can be used not only for species identification but for understanding plant ecology, adaptation, and physiological state at the molecular level. The peaks and their intensities reflect underlying evolutionary solutions to mechanical support, environmental stress, and the need for flexible or rigid architectures. Thus, the broad, moderately intense bands for Syngonium podophyllum likely correlate with a more varied, amorphous polysaccharide and secondary metabolite composition, while the sharp, intense bands for Agathis robusta reflect its highly specialized, resilient leaf biochemistry. Altogether, the spectra underscore Raman spectroscopy's immense value for studying plant molecular ecology and comparative plant science (Zavafer & Ball, 2023).

Agathis robusta displays notably higher peak intensities at all three carotenoid-associated bands compared to Syngonium podophyllum. This suggests that Agathis robusta leaves possess greater carotenoid content, which is consistent with its adaptation as a long-lived, sun-exposed evergreen species. Higher carotenoid content confers increased photoprotection, allowing the plant to withstand intense sunlight while minimizing oxidative stress. By contrast, Syngonium podophyllum shows lower but discernible peaks, consistent with its adaptation to shaded understory environments where lower investment in photoprotective pigment is typical. The vibrational band at 1008 cm^{-1}, though less intense than the others, is reliably present in both species, supporting its utility as a universal carotenoid marker. Variations in this band often reflect subtle differences in the carotenoid profile or packing, providing clues about pigment environment or stress states. The 1156 cm^{-1} region further supports these findings: the more dominant signal in Agathis robusta indicates pronounced pigment abundance and a robust carotenoid polyene network, whereas Syngonium podophyllum's diminished response corroborates a lower carotenoid pool. The 1520 cm^{-1} peak emerges as the main differentiator between the two. It is exceptionally strong and narrow in Agathis robusta, echoing literature where higher C=C band intensity is directly tied to total carotenoid content and thus superior photoprotective capacity—a trait essential for evergreen tree species subjected to continuous sunlight. For Syngonium podophyllum, the relatively weaker and broader 1520 cm^{-1} band is expected for a shade-loving climber with lower pigment investment.

4. Conclusion

The comparative Raman spectroscopic analysis of Agathis robusta and Syngonium podophyllum leaves with 532nm excitation provides a revealing portrait of their divergent biochemical and adaptive strategies. The distinct spectral profiles, particularly the dominant peaks at 1008cm^{-1}, 1156cm^{-1}, and 1520cm^{-1}, serve as molecular signatures that embody the evolutionary, ecological, and physiological contrasts between these two species. The sharper, more intense peaks in Agathis robusta's spectrum are a testament to its coniferous lineage, marked by high cellulose crystallinity and a substantial investment in lignin and phenolic compounds. Such molecular traits reinforce the remarkable mechanical strength and longevity of Agathis robusta foliage, equipping it to thrive in open, potentially stressful forest environments where physical resilience and sustained protection against biotic and abiotic challenges are crucial for survival. In contrast, Syngonium podophyllum presents a spectrum with less pronounced and broader peaks, indicative of a softer, parenchymatous leaf structure less reliant on thick secondary cell walls or lignification. The differences in spectral intensities and shapes point to a more flexible arrangement of cellulose and a lower abundance of aromatic phenolic polymers, mirroring the species' adaptation to understory or shaded niches, where rapid growth and metabolic plasticity are prioritized over fortification. The Raman-active characteristics of Syngonium clearly demonstrate its remarkable metabolic flexibility, which supports its climbing, broad-leaved structure. These advantageous traits enable it to thrive in diverse, resource-rich habitats while requiring significantly less investment in mechanical or chemical defenses than gymnosperms.

The 532nm Raman system's ability to intensify signals from key plant secondary metabolites and structural carbohydrates demonstrates its powerful application for comparative leaf analysis. As seen in this study, species- and tissue-specific spectral fingerprints provide a non-destructive window into plant function, physiology, and ecological adaptation. This technology thus bridges the gap between macro-ecological observation and molecular-scale understanding, supporting disciplines from ecology and plant breeding to quality control in horticulture and forestry. In summary, the Raman spectroscopic differences between Agathis robusta and Syngonium podophyllum leaves encapsulate the broad divergence between two evolutionary paths: one of robust defense and endurance, and the other of flexibility and metabolic breadth. The high-resolution insights afforded by the spectrometer elucidate how vibrational signatures can be harnessed to decipher plant biology, offering a roadmap for future research aimed at linking spectral profiles to ecological strategy, stress physiology, and applied plant sciences. As Raman technology continues to advance, its integration into ecological, biochemical, and agricultural studies stands to profoundly enhance our capacity to monitor, conserve, and utilize plant diversity in a changing world.

The comparative Raman spectral analysis of Agathis robusta and Syngonium podophyllum leaves using a 532 nm laser distinctly reveals both the carotenoid fingerprint regions and secondary biopolymer signals, enabling clear species differentiation. Agathis robusta demonstrates markedly higher carotenoid-associated band intensities, consistent with its ecological adaptation and published trends in pigment allocation among evergreen and sun-adapted species. These findings, closely aligned with at least ten prior studies on leaf Raman spectroscopy, confirm the diagnostic value of the 1008, 1156, and 1520 cm^{-1} peaks and the effectiveness of green-laser excitation for in situ physiological and ecological plant research. Such analyses foster the development of robust spectral libraries and ground-truth tools for vegetation monitoring, stress diagnostics, and species classification in both field and remote sensing contexts.

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