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REVIEW ARTICLE

Review–Vibrational spectroscopy-aided diagnosis, prognosis and treatment of inflammatory bowel disease

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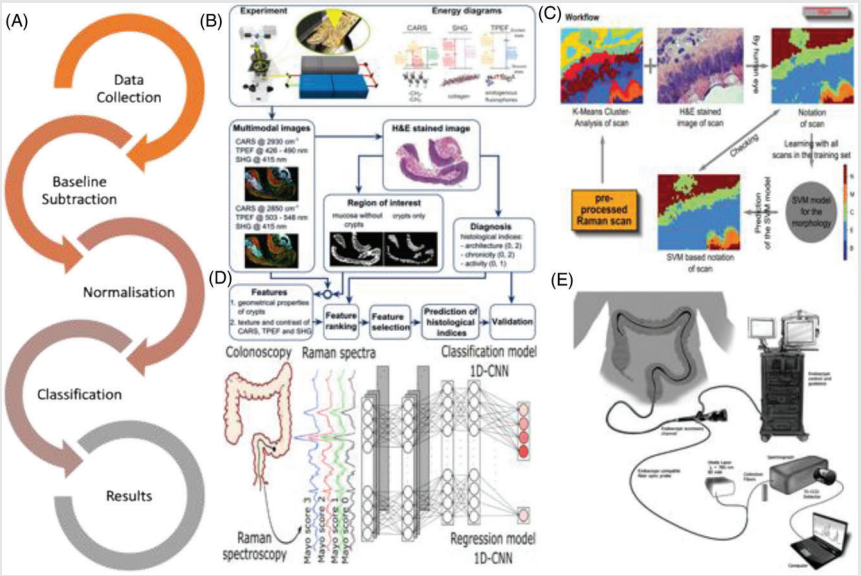
Graphical Abstract



Inflammatory bowel disease (IBD) is a group of chronic inflammatory intestinal conditions that affect an estimated 10 million people worldwide, and currently, there is no known cure for the disease. This review is focussed on the potential of Raman spectroscopy (RS) in the diagnosis and differentiation of IBD as well as the disease-indicative biomarkers, overviewing the different Raman methodologies currently applied within clinical IBD research as well as their ability to act as monitoring and therapeutic tools in the future. Additional synopsis includes the use of RS in the identification of biomarkers and how these may contribute to the non-invasive and early detection of IBD, with further in-depth summary of the primary methodologies applied in Raman-IBD analyses and a comprehensive spectral library with corresponding peak assignments from the current Raman-IBD literature, collectively, laying a platform for the introduction of new tools for advancing the understanding of the overall biochemical changes of IBDs and the development of timely diagnostic and therapeutic approaches.

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Abstract

Background: Inflammatory bowel disease (IBD) represents a group of chronic gastrointestinal disorders characterised by complex and multifaceted pathologies. The pursuit of non-invasive, accurate and rapid diagnostic methods and therapeutic monitoring tools has led to the emergence of Raman spectroscopy (RS) as a promising analytical technique in the field of IBD. RS offers molecular specificity with the potential to uncover valuable insights of the pathophysiology of IBD, posing it as a rapidly growing field of interest in both research and clinical settings.

Main: This comprehensive review aims to provide an overview of the current state of research in the field of IBD-RS, with an emphasis on its potential diagnostic, prognostic, and therapeutic monitoring capabilities. We explore the molecular characterisation of bodily fluids including blood, urine and saliva and the ability of RS to rapidly determine the underpinning biochemical changes in their composition. The review also highlights recent studies and advances, that have employed RS to differentiate between healthy and diseased states, classify the IBD subtypes, identify candidate biomarkers and to monitor the therapeutic response to treatment in the form of mucosal healing. Additionally, we comment on the challenges and limitations associated with current research as well as with the clinical translation of RS for IBD diagnosis.

Conclusions: As the field of vibrational spectroscopies continues to evolve particularly in the broader IBD context, to aid the diagnosis, prognosis and treatment, this review serves as a valuable resource for clinicians and researchers alike, examining the potential of Raman-based spectroscopy and the emerging technologies to advance the understanding and triaging of IBD. Ultimately, the integration of RS into everyday clinical practice holds promise for more effective management of IBDs, addressing the major unmet need in the fields of gastroenterology and of clinical medicine as a whole.

KEYWORDS
artificial neural network classification, diagnostics, inflammatory bowel disease, monitoring, Raman spectroscopy

1 | BACKGROUND

Inflammatory bowel disease (IBD) is a group of chronic inflammatory intestinal conditions that affects an estimated 10 million people worldwide.[1,2] The incidence and prevalence of IBD vary considerably both between and within geographic regions, with North America and Northern Europe accounting for the highest incidence at 15.4 people per 100 000 and 23.1 per 100 000.[3] Over time, the prevalence of IBD is increasing primarily due to the chronic condition, which lacks a definitive cure. Further factors contributing to this trend include the young age of onset, relatively low mortality rates and the potential for exponential growth due to increasing incidence rates, as well as an aging population.[4] IBD is comprised of two main conditions: ulcerative colitis (UC) and Crohn's disease (CD). Although these cause inflammation in the digestive system, UC affects the large intestine, while CD can affect any part of the digestive tract from the mouth to the rectum. These complex illnesses are characterised by both acute and chronic disease states with a relapsing-remitting pattern.[5] However, IBD usually requires life-long treatment. Currently, there is no known cure for the disease. Initial signs of IBD often appear upon exposure to medications or an infection that irritates the intestines, with common symptoms including abdominal pain, diarrhoea (oftentimes with blood), faecal incontinence, rectal bleeding, weight loss and malnutrition. Inflammation of the intestines can also cause swelling or masses, and if uncontrolled, IBD can damage the intestine causing abscesses, strictures and fistulas with the long-term complications leading to an increased risk of colon cancer.

IBD typically presents as a sudden flare-up symptom, with the majority of individuals diagnosed under the age of 35. At present, there is no single test to diagnose IBD, as the symptoms of the disease significantly overlap with other conditions including infections and other digestive system disorders.[6] Thus, a typical IBD diagnosis comprises a combination of a physical exam, colonoscopy, upper endoscopy with biopsies, stool sample analysis, imaging (computerised tomography [CT], magnetic resonance imaging [MRI] or X-rays) and blood work such as anti-*Saccharomyces cerevisiae* antibody (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA) antibodies.[7,8] Current diagnostics of IBD are associated with certain challenges such as, the 'gold standard' colonoscopy and endoscopy being invasive, time-consuming, not timely enough and may risk infection in patients. Moreover, distinct morphological or structural abnormalities may not be apparent in early pathologies.[9] Blood and stool tests often lack sensitivity and specificity and may fail to detect mild or early-stage disease.[7,10,11] Furthermore, imaging, such as MRI and CT, entails high costs, placing a burden on healthcare systems, with access to such specialised tools and the required expertise often limited, particularly in remote geographical locations, causing delays in diagnosis and treatment.

Early and accurate detection of IBD is crucial in providing appropriate interventions. It can further facilitate monitoring the progression of disease as well as the therapeutic responses. However, timely diagnosis of IBD still remains an unmet need. With an overlap in presentation, symptoms and disease progression, discrimination of UC from CD is essential when selecting the most appropriate therapeutic or surgical regimen to improve patient prognosis. The determination of the correct therapeutic regime is often evaluated based on the severity of active inflammation, which is often further complicated by the lack of recognised gold standards for accurate diagnosis.[12] Clinically, IBD diagnosis is based on a combination of symptom presentation, histological and endoscopic evaluation of the mucosa.[13] This diagnostic process is both time-consuming and highly invasive. Moreover, endoscopic evaluation of chronic colitis varies between CD and UC, with 89% of patients with endoscopists often facing a disease with a non-differentiating appearance and histopathologists one of inflammation consistent with both forms of the disease.[9,13] This further highlights the need for non-invasive, rapid and accurate tools to reduce the burden of disease diagnosis as well as successfully differentiate UC from CD.

Raman spectroscopy (RS) has been emerging as a potential analytical tool with many ramifications, particularly for rapid, non-invasive and specific disease diagnostics.[14–21] RS is a non-destructive, label-free technique whereby scattered light is used to measure the vibrational modes of a molecule of interest.[22] Originally focused on the characterisation of various analytes in the fields of chemistry and physics, RS has recently been demonstrating unprecedented applications in clinical research ranging from cancer,[18,23–25] infectious diseases[15,26,27] and neurodegenerative diseases.[28] Herein, the predominant

focus is on the potential of RS in the diagnosis and differentiation of IBD as well as the disease-indicative biomarkers. We provide an overview of the different Raman methodologies currently applied within clinical IBD research as well as their ability to act as monitoring and therapeutic tools in the future. Additional synopsis includes the use of RS in the identification of biomarkers and how these may contribute to the non-invasive and early detection of IBD. An in-depth summary of the primary methodologies applied in Raman-IBD analyses, along with a comprehensive spectral library with corresponding peak assignments from the current Raman-IBD literature, are further overviewed and generated. These lay a platform for the introduction of new tools for advancing the understanding of the overall biochemical changes of IBDs and the development of timely diagnostic and therapeutic approaches. Furthermore, it would enable improving the clinical management of patients, as in the case of an evolving and multifaceted pathology such as IBDs, RS may provide integrative and complementary clinically useful information, or be useful at different time points or in different settings.

2 | VIBRATIONAL SPECTROSCOPY

The first observation of Raman scattering was in 1928 by C.V. Raman and K.S. Krishnan.[22,29] Although industrial laboratories have used RS for many years, since the early 1980s, interest has grown exponentially due to technical advances in instrumentation and reduced costs. Raman is a versatile technique suitable for the analysis of a wide range of samples across various fields including physics, chemistry, engineering, materials science, biology and medicine.[15,30–32] RS resolves many of the limitations associated with other spectroscopic techniques and is both a qualitative as well as quantitative tool. By measuring the frequency of scattered photons, the technique can be described as qualitative, while measuring the intensity of the scattered photons enables quantitative analysis.[33,34] In RS, the sample is typically illuminated with a monochromatic laser beam, which interacts with the molecules within the sample and, thus, scatters the incident light.[35] In the majority of the scattering events, upon its interaction with the photon, the energy of the molecule remains unchanged, therefore, the energy (or wavelength) of the scattered photon is equal to that of the incident photon. This is called elastic or Rayleigh scattering. In a much rarer event (approximately 1 in a million photons), Raman scattering occurs, where the energy of the scattered light is changed by a specific amount depending on the chemical bond within the molecule that has interacted with the photon. RS is based on this effect, where the frequency of a small portion of the scattered light, being at a different frequency from the monochromatic incident light, is used to measure the vibrational modes of the sample and can provide both bio-chemical and structural information as well as the specific identification of substances through the characteristic spectral 'fingerprint'.

A typical diagnosis of IBD currently involves a combination of medical history, physical examination as well as blood and stool tests with a subsequent endoscopy and histopathological analysis. However, these procedures are invasive, time-consuming, not timely enough and may risk infection in patients. Moreover, distinct morphological or structural abnormalities may not be apparent in early pathologies.[9] RS, thus, may provide a useful tool in clinical settings to diagnose IBDs in a timely manner via detection of altered biochemistry within an individual's body, specific to particular disease states that precede macroscopic tissue changes.[36] RS can not only rapidly provide information on the molecular structure and composition of a sample, which can be used to identify specific biomolecules, but also rapidly yield quantitative information about the concentration of the detected bio-analytes, which are highly useful for both monitoring disease progression as well as patients' responses to treatments. Spectroscopic 'fingerprints' of Raman data have been successfully shown to identify the disease states of the subject from which the biofluid has been collected, and in contrast to the in-vitro bioassays, the availability of inexpensive, portable Raman instruments makes this technique particularly attractive for point-of-care, rapid sampling, analysis and screening of biofluids and tissue. The potential of RS in the ability to monitor IBD in clinical settings, due to its high sensitivity and label-free methodology, can further accelerate the discovery of potential biomarkers of disease, meaning the subsequent screening is simpler, quicker and less expensive. It is important to note that although the ability of RS to rapidly and accurately identify target molecules renders it a valuable technique, analysing biological samples introduces an added layer of complexity. Since biological systems and samples are typically composed of a broad array of biochemicals including proteins, carbohydrates, lipids, etc. Thus, spectra from biological specimens, such as tissue and biofluids, may appear highly similar, as vibrations from each of these molecules will be present in the Raman spectra. Analyses of these, can subsequently, not be straightforward to untrained personnel. Therefore, the combination of RS with subsequent computational algorithms enables it to rapidly and accurately decompose the signal or separate the data according to statistical properties inherent in the dataset using various multivariate techniques, discussed in more detail below. This yields an overall powerful tool to provide meaningful analysis from highly specific spectra of biological samples,

which, together with generated spectral libraries of peak assignments, establish important biochemical 'fingerprints' as characteristic barcodes providing a broad range of information pertaining to bio-chemical processes and pathways of diseases to be explored.

RS, although a powerful technique for analysing various biofluids, is associated with some limitations. These include fluorescence interference, known to occasionally overwhelm the Raman signal and obscure spectral signatures; sample complexity—where untangling the Raman spectra of complex biological samples can be challenging, the cost of specialised equipment, and low sensitivity due to Raman scattering being an inherently weak process, thus, rendering it difficult to detect low concentrations of biomolecules. Nevertheless, RS is continuously optimised and remains a valuable tool for the analysis of biofluids. On-going research aims to address these challenges via a combination of advancements in instrumentation, data analysis techniques and the development of enhanced Raman probes and substrates. One such development is known as surface- enhanced RS (SERS).

With the advent of SERS and the subsequent enhancement of the Raman signal of up to 10^{11} times, single-molecule detection can be achieved.[37,38] SERS typically uses nano-scale surfaces of gold or silver to induce a highly localised electromagnetic field by surface-confined, laser excitations.[39–41] A substantial enhancement in the Raman signal is observed when a molecule is absorbed or within 10 nm of the surface. For instance, analysis of plasma via conventional RS reflects different proteins yet, the smaller molecules remain almost undetectable, however, via SERS, a considerably amplified signal is produced, enabling the detection of *minute* biomolecule levels.[42–48] SERS, therefore, exhibits several advantages, including high sensitivity and selectivity with the ability to detect analytes down to the attomolar level. It further has a relatively low sensitivity to water, rendering it suitable for in-situ and in-vitro applications of biological samples. Overall, SERS is well-suited to an extensive scope of applications ranging from surface chemistry, catalysis, food science and pharmaceuticals.[42,46,48–52] However, despite these advantages, SERS has often been underutilised due to challenges in reproducibility, high costs associated with the development of suitable substrates and the need for highly specialised equipment and expertise.[39,42]

There are other available vibrational spectroscopy types, such as the infrared spectroscopy (IR), offering an additional complementary technique. IR detects the absorption of light by a compound and is dependent on the energy absorbance taking place between different vibrational and rotational states of the molecular bonds.[53–56] In recent years, IR methodologies have greatly advanced clinical medicine. Of a particular interest is the emerging IR technology, the attenuated total reflectance Fourier transform (ATR-FTIR) spectroscopy, commonly applied in the analysis of blood serum, blood plasma, urine and saliva.[54,56–60] ATR-FTIR allows the direct examination of solid or liquid samples without further preparation, it is non-destructive, delivers high reproducibility and provides a rapid diagnostic alternative with output results within seconds to minutes. Despite this, ATR-FTIR has the drawback of spectral artifacts, where it is affected by factors such as crystal temperature, pressure and contact force.

3 | LITERATURE OVERVIEW

The literature review was performed using PubMed and Elsevier (ScienceDirect) from Inception up to August 2023, identifying studies applying RS in IBD. Search strategies included the following combinations: (inflammatory bowel disease) OR (Crohn's disease) OR (ulcerative colitis) AND (Raman Spectroscopy). Excluded are studies including animal models. Included are studies that used RS in either the diagnosis and prediction of IBD or the assessment of the severity of IBD or clinical outcomes in IBD and prediction of therapeutic response in IBD. The search strategy highlighted 21 studies of interest using RS and IBD, of which 17 studies met all inclusion criteria, and therefore, were included in the review (Figure 1).

Of the identified studies, 56% (9/16) were published within the last 5 years (>2018). All 16 studies were performed on human tissue or biofluid, with 9 performed on tissue, 3 with urine, 1 with saliva, 2 with plasma and 1 with faecal samples. In addition, 10 of the studies focused on disease diagnosis, 2 evaluated mucosal healing (MH), 2 investigated disease severity, 1 aimed to identify perianal fistulas in CD patients and 1 focused on the identification of potential biomarkers of IBD. There were a further seven studies which used mixed IBD cohorts (UC and CD) with five focused only on UC and four on only CD (Table 1).

4 | METHODOLOGY

4.1 | Raman spectroscopy setup

Of the studies identified, six used custom-built Raman set-ups, while 10 used lab-based commercial systems. Most of the Raman set-ups exploited an excitation laser wavelength of 785 nm, except Addis et al., who used 514 nm and Chemavaiska et al., a 532 nm laser.[61,62] Due to the efficiency of Raman scattering, scaling inversely with excitation wavelength to the fourth power, there has been a movement towards shorter excitation wavelengths, which

TABLE 1 Overview of publications applying Raman spectroscopy in diagnosis, treatment, or biomarker discovery of IBD.

			Patient Cohorts			Raman		Performance		
Publication	**Application**	**Sample Type**	**UC**	**CD**	**HC**	**Laser (nm)**	**AI Classification**	**Sensitivity (%)**	**Specificity (%)**	**Accuracy (%)**
Pence et al., 2017[12]	Diagnosis of IBD	Tissue	8	15	8	785	SMLR	62-86.2	22.9-74.5	–
Smith et al., 2021[64]	Mucosal healing and patient response to biologics	Tissue	42 6	32 8	-9	785	SKiNET	96.3	95	95.6
		Tissue	6	8	9			96.2	88	91.6
Acri et al., 2020[65]	Diagnosis of IBD in paediatric patients	Faecal	9	15	19	785	ROC, Youden Index	–	–	–
Bielecki et al., 2012[13]	Discrimination of IBD	Tissue	13	14	–	785	PCA, K-means clustering	93.84	90.3	88.26
Kirchberger-Tolstik et al., 2020[36]	Assessment of disease severity	Tissue	140	–	–	785	1D-CNN	78	93	–
Ding et al., 2017[66]	Determination of histological inflammatory status in vivo	Tissue	18	–	31	785	Statistical analysis—Tukey honest significant difference	83.5	97.1	–
Addis et al., 2016[61]	Assessment of mucosal healing	Tissue	60	–	–	514	Linear regression analysis	–	–	–
Tefas et al., 2020[67]	Diagnosis of UC	Plasma	28	–	35	785	PCA-LDA	86	92	89
							PLS-DA	89	94	92
Morasso et al., 2020[9]	Diagnosis of CD	Plasma	–	77	45	785	PCA-LDA	80	85.7	83.6
Li et al., 2021[45]	Diagnosis of CD	Urine	–	98	45	785	PCA-LDA	–	–	69.9
Zhu et al., 2023[68]	Perianal fistula in CD patients' identification for treatment strategies	Urine	–	110	–	785	PCA-SVM	71.43	80	75.71
Bi et al. 2011[69]	Discrimination of IBD	Tissue	12	9	–	785	–	–	–	–
Veenstra et al., 2014[70]	Diagnosis of UC	Tissue	4	–	–	785	PCA	82	89	
								87	93	
Wu et al., 2022[71]	Discriminating active CD from inactive CD	Urine	–	100	88	785	PCA-SVM	–	–	63.6
Chernavskaia et al., 2016[62]	Identification of disease severity	Tissue	6	7	7	532	Multimodal Imaging	–	–	–
Buchan et al., 2023	Identification of potential biomarkers of IBD	Saliva	26	25	50	785	SKiNET	–	–	98.2

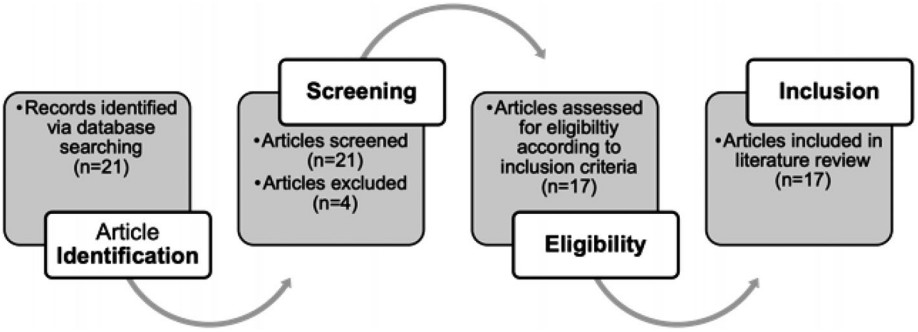

FIGURE 1 Workflow used to identify and assess literature suitability.

typically result in stronger signal, enhancing sensitivity and enabling detection of trace analytes.

However, shorter wavelengths are more likely to result in sample fluorescence, complicating sufficient Raman signal, detection and analysis. Hence, the 785 nm excitation is the most frequently used in Raman-IBD studies since it reduces the fluorescence. Furthermore, at this longer wavelength, the light is capable of penetrating deeper into thick or turbid samples, thus, making it suitable for in vivo or non-destructive analysis of biological tissue, with the lower-energy photons at near-infrared excitation are less likely to cause photodamage to sensitive biological samples.[63]

Across the Raman-IBD studies, a range of different laser powers and optics are employed, ranging from 10 mW by Smith et al. up to 90 mW by Morasso et al.[9,64,72] The choice of laser power in RS requires establishing a balance between maximising the signal intensity and minimising the potential sample damage or interference. It depends upon the nature of the sample, the desired signal strength and the potential for fluorescence. In the selected studies, typically those involving tissue samples, RS was employed using the lower powers and shorter exposure times than those involving plasma, urine or faeces. For instance, Pence et al. set an exposure time of 250 ms, while Addis et al. applied a 60 s exposure to faecal samples, with the former stating they were unable to increase integration times due to a substantial background noise and autofluorescence.[12,61] Choosing an adequate laser power is known to improve the signal-to-noise (SNR), which is in turn, central to obtaining high-quality spectra. The higher the SNR, the greater the overall accuracy and precision of spectral measurements and data analysis. Laser power also influences the depth from which Raman signals can be obtained.[72] Higher power generally penetrates deeper into the samples, allowing for the analysis of subsurface layers of thicker samples, particularly important for applications with in-vivo tissue analysis, as observed by both Pence et al. and Ding et al., who examined colon tissue in-vivo using portable, custom-designed Raman systems.[12,66] Higher laser power can, however, lead to sample damage, particularly for sensitive, biological samples. This photodamage or degradation can occur, leading to sample alteration and the introduction of artificial carbon D and G spectral bands. Optimisation of Raman setup, therefore, requires careful empirical testing and adjustments for each unique experimental setup and sample type.

4.2 | Raman spectral pre-processing

Generally, in Raman-IBD studies, a form of spectral pre-processing is applied including baseline subtraction, normalisation or cosmic ray removal. First, in many Raman spectrometers, baseline shifts exist, whereby the generated spectra contain both the desired signals as well as the more undesirable elements including background noise. Many methods of baseline subtraction can be applied, such as asymmetric least squares (AsLS), polynomial fitting, differencing and filtering, with the chosen method highly dependent upon the level of precision required, patterns and obtained computational times.[73,74] Polynomial fitting methods, for example, are a simple and effective way to remove baseline artifacts caused by factors such as instrumental drift, scattering, sample thickness or sample inhomogeneity. The method involves manually identifying the areas in the spectrum that contain undesirable or non-Raman-like locations and estimating this background by way of a polynomial function. The degree of polynomial is dependent on the overall complexity of the baseline, with the most commonly applied fits being linear (1st degree), quadratic (2nd degree) or cubic (3rd degree) polynomials. The selected fit is then applied to spectral areas with no features of interest and subsequently subtracted from the raw spectrum point-by-point, leaving behind a baseline-corrected spectrum.[74,75] Polynomial baseline subtraction enhances the clarity of spectral features and aids in the interpretation and analysis of spectral data. AsLS, on the other hand, combines a smoothing filter with asymmetric weighting deviations from the determined smooth

trend in order to develop an effective baseline subtraction method.[73,76] This method has the drawback associated with the smoothness constraint in that it only considers the second derivative. In most studies, baseline subtraction is performed using a high-order polynomial fit (4th or 5th) while, Kirchberger-Tolstik et al. employed an AsLS method and Ding et al. used a cubic spline interpolation.[36,66]

Subsequently, normalisation is routinely performed to account for intensity variabilities due to factors such as sample concentration variability, laser power fluctuations and differences between sample set ups.[77,78] Commonly employed normalisation methods in RS include scaling—where the minimum intensity is set as 0 and the maximum as 1 and all values scaled proportionally, *Z*-score normalisation—where the data have a mean of 0 and a standard deviation of 1 to centre the data; and vector normalisation with the spectrum being scaled by dividing it by its own vector length, ensuring that the normalised spectra have a final magnitude of 1.[77] Normalisation using area under the curve has been used by Addis et al., with five studies using vector normalisation.[9,13,36,45]

In addition to baseline subtraction and normalisation, smoothing is often used to overcome the inherent issue of noise in Raman spectra. Smoothing is used to remove high-frequency components from the spectra to reduce noise and simplify the identification of important spectral features.[79] The most used smoothing filter is the Savitsky-Golay filter, where a polynomial regression is applied to the data, that is, a polynomial curve is fitted to small segments or peaks within the Raman spectrum, with the smoothed curve providing an estimate as to the underlying Raman signal.[79,80] The use of a Savitsky-Golay filter for spectral smoothing was employed by three of the studies,[12,61,66] with only Addis et al. indicating a filter smoothing width of 9 and a polynomial order of 3.[61] Due to the differences in spectral acquisition and Raman setup, each study required the pre-processing techniques used to be applied in a specific order. The most applied procedure included baseline subtraction, followed by normalisation leading to data classification (Figure 2).

Spectral features were further compared and classified using various statistical methods, which provide valuable information regarding spectral differences or relationships between the data sets, in combination with traditional pre-processing methods. The majority of studies employed either Student's *t-test* or Wilcoxon-Mann-Whitney *U test*.

4.3 | Classification versus molecular characterisation

The studies identified within this review predominantly focus on using RS for either classification or molecular characterisation of spectral data. While both are essential for the comprehensive understanding of IBD and its potential translation into clinical fields, they each serve a different purpose and apply diverse methodologies. Raman characterisation focuses on identifying and understanding the molecular composition and the associated chemical properties of the biofluid or tissue under investigation. This approach classically involves detecting and quantifying specific molecules, chemical bonds or structures within the sample. Characterisation aims to provide detailed information as to the sample's biochemical makeup, identifying biomolecules including lipids, proteins, and nucleic acids whilst also assessing changes to the molecular constituents such as protein structure and confirmation or lipid profiles in samples where disease is present. Commonly, the methodology involves using RS to obtain spectra, which serve as chemical fingerprints, with subsequent analysis of spectral peak intensities and Raman shifts used to identify specific molecular vibrations and chemical bonds, thus, allowing for the determination of the composition and structural characteristics of the biofluid. This approach was employed by Ding et al., Addis et al., Chernavskaia et al., Bi et al. and Wu et al.,[12,61,62,66,69,71] where the authors applied statistical methods including the parametric (*t-test*). Shapiro W ilk and Kolmogorov-Smirnov statistical analysis were employed by Wu et al., while Addis et al. applied the statistical Mann-Whitney U test and Ding et al. investigated the receiver operator curve (ROC). These outputs provided disease-related biochemical changes associated with IBD.

Conversely, RS classification focuses on using the spectral information obtained from the analysis to classify biofluid or tissue samples into different categories or states such as healthy or diseased. Such classification usually involves the development of models or algorithms capable of differentiating between the data classes. Machine learning techniques are most routinely used to train the classifiers and label the data sets accordingly. Of the studies identified, 10 have[9,12,13,36,45,64,65,67,68,70] applied a variation of multivariate analysis or machine learning to train their models using spectral data from a set of known biofluid samples including primarily healthy and diseased classes, MH and inflammation or disease severity. Several further researchers have developed and applied further forms of supervised machine learning approaches including the artificial neural networks (ANNs) by Smith et al., Buchan et al. and Kirchberger-Tolstik et al.[36,64] Other studies harnessed dimensionality reduction and feature extraction methods, such as principal component analysis-linear discriminant analysis (PCA-LDA),[9,45,67] partial least squares-discriminant analysis (PLS-DA)[67] or principal component analysis-support vector machine (PCA-SVM).[68,71] In each instance, the goal was to provide a diagnostic tool which

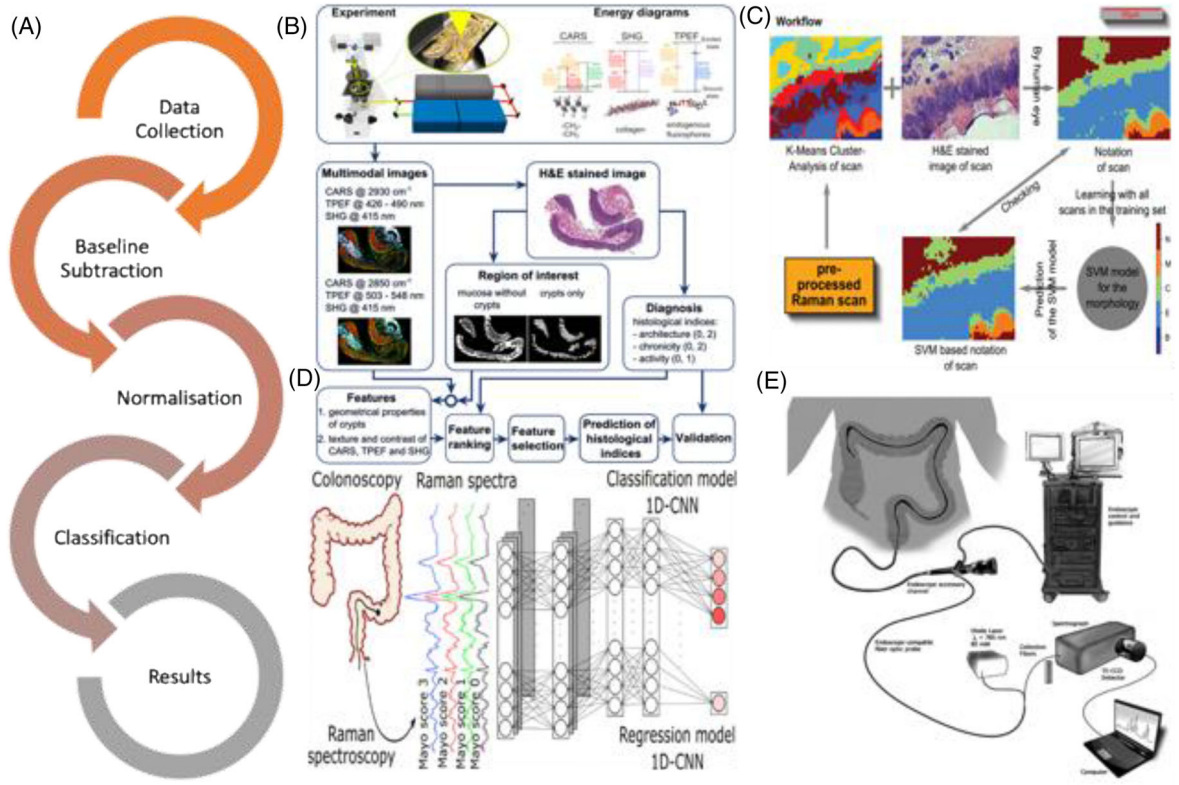

FIGURE 2 (A) Overview of a typical procedure for Raman data analysis and processing. (B) Workflow used in *real-time* endoscopic assessment of IBD disease activity including initial multimodal imaging of CARS, TPEF, SHG and H and E staining closely examined by pathologist and feature extraction of regions of interest to discriminate both morphology and intensity related to diagnostic criteria.[62] (C) Overall work path in the classification of IBD based on imaging of epithelium cells consisting of *k*-means clustering of morphological features, followed by SVM learning and subsequent prediction via a 10-fold cross-validation with comparisons made to those identified by the pathologist as well as with H and E staining.[13] (D) Schematic representation of the methodology used by Kirchberger-Tolstik et al.[36] comparing Raman spectra to Mayo endoscopic scores in UC.[36] (E) Illustration of an integrated Raman set-up and endoscope instrumentation for in-vivo analysis used by Pence et al.[12] Reprinted with permission from [12, 13, 36 and 62].

could rapidly and accurately identify the presence or absence of IBD based on the obtained spectral signature. Overall, both approaches complement one another and are vital to gain a comprehensive understanding as to the disease development and diagnosis using RS.

4.4 | AI/Artificial intelligence/Artificial neural network multivariate data classification

Most of the studies employ multivariate analysis to investigate spectral changes across the whole spectrum. Notably, Ding et al. and Acri et al. applied somewhat different approaches using the Tukey honest significance variance and receiver operator curve (ROC), respectively.[65,66] Multivariate analysis of RS data enables powerful extraction of meaningful information from complex high-dimensional, spectral datasets with multiple variables.[81] It also enables the detection of subtle spectral shifts, which would be otherwise difficult to differentiate using traditional methods such as manual peak selecting approaches. Frequently applied PCA can reduce the dimensionality of data while preserving the relevant information. Techniques, such as linear discriminant analysis or support vector machines employed by Tefas et al., Li et al. and Morasso et al., can classify samples into pre-defined groups such as, IBD and healthy controls (HCs), based on their spectral patterns, which is particularly useful in sample identification and disease diagnosis.[9,45,67] Approaches, such as PLS regression, used by Tefas et al., are also capable of establishing quantitative relationships between spectral data and sample properties, valuable for the accurate and rapid quantification of biomarkers in a dataset.[67] Machine learning, a sub-field of artificial intelligence (AI), is another approach applied in studies by Smith et al and Buchan et al.[64] In these studies, a novel supervised machine learning algorithm uses an ANNs inspired by the structure and functioning neural networks such as the brain. They consist of interconnected nodes, termed artificial neurons or nodes, organised into layers.[82] The power of ANNs lies in their ability to learn complex

patterns and representations from data, thus, presenting a valuable decision support tool to aid in medical diagnosis. It is based on self-organising maps with self-optimizing Kohonen index network (SKiNET) as a framework for multivariate analysis that simultaneously provides (i) dimensionality reduction, (ii) feature extraction and (iii) multiclass classification. SKiNET performs visual separation to identify the underlying chemical differences between classes, providing accurate classification for simultaneously rich-information and high-classification specificity even for low laser powers and short acquisition times, representative of the real-world point-of-care conditions.

Machine learning applied in these studies is essential for the clinical translation of RS-based disease classification, enabling accurate, automated, and scalable disease diagnosis whilst enhancing objectivity and reproducibility. It can also be integrated into RS systems and methodologies as well as clinical workflows, thus, improving time to results as well as patient care and long-term outcomes. It also plays a crucial role in realising the full potential of RS in clinical applications and beyond. Although the focus here is on IBDs, further afield similar approaches have been used in cancer diagnostics, where for instance, Hernándes-Vidales et al. used PCA-SVM to distinguish between biomarkers of cancer with a high accuracy of 94%, highlighting its suitability for the investigation of various forms of cancer.[83] Similarly, Mehta et al. applied PCA-LDA to differentiate blood serum from control and meningioma patients, yielding 92% classification accuracy.[84] This was further expanded to cervical cancer diagnosis, where Daniel et al. used near-IR RS combined with ANN to determine biochemical changes associated with cancerous cells, successfully achieving 99% accuracy.[85]

5 | APPLICATIONS

5.1 | IBD diagnosis and discrimination

Raman spectroscopic-based approaches have been employed in a wide array of diagnostic applications, aiming to provide objective and *real-time* assessments of diseases.[17,28,86,87] There has been a notable increase in interest in the use of Raman analysis for the study of IBDs in recent years, indicating that RS can enhance diagnostic accuracy due to the detailed molecular information it is capable of uncovering in a rapid manner. In the field of IBDs, whilst several researchers aimed to identify either CD or UC from patient samples,[13,69] others aimed to investigate and discriminate both UC and CD from healthy patient samples[12,62,64–65] (Figure 3).

One of the earliest studies published in this field by Bi et al. examines the structural and compositional changes of both UC and CD colon tissue specimens to differentiate between the two IBD subtypes. The authors used a custom-designed Raman fibre-optic probe to analyse the tissue samples in-vitro. The probe was placed in contact with the mucosal surface of the sample for 3 s to obtain spectra.[69] After statistical analysis, closely examining both the spectral peak differences and molecular origins, a significant difference was observed between the nucleic acid, phenylalanine and lipid regions of the spectra. Assignments for the identified Raman bands are summarised in Supporting Information Table S1. This study importantly demonstrated a higher lipid content with a lower phenylalanine and nucleic acid content in UC tissue samples, indicating the potential of these characteristics in disease discrimination. The significant variation noted in lipid content is most likely due to CD, often presenting with transmural inflammation, which involves all layers of the bowel wall, including the sub-mucosa and therefore, brings about changes in the lipid content due to inflammation, fibrosis, and the formation of granulomas. UC, on the other hand, primarily involves inflammation of the mucosal layer thus, the sub-mucosal changes are generally less pronounced than in CD. To quantify and compare these changes, further studies, such as, histological examination or lipidomic analyses, would be required. Limitations of this study include the exclusion of HCs and the use of statistical chemometric methods only. The inclusion of multivariate analysis could further offer the advantages of dimensionality reduction, pattern recognition and effective visualisation.

Bielecki et al. reported a Raman microspectroscopic approach to diagnose IBD. The authors first collected Raman measurements and morphological images (obtained via haematoxylin and eosin staining) from UC, CD and HC specimens.[13] Subsequently, a unique classification methodology, a support vector machine, capable of analysing histological and spectral data to identify and visualise the tissue morphology and ultimately differentiate the epithelial structures from connective tissue and mucus, was employed. This approach demonstrated significant differences between each of the three groups (UC, CD and HC) with the classifier achieving an accuracy of 98.9%, paving the way for the automatic and objective classification of IBD via RS even in patients with minimal or moderate inflammation. Significant changes were observed in the heme bands, which were associated with increased levels of inflammation in IBD patients compared to HC. Additionally, UC is distinguished by a distinct hyperaemic colonic mucosa, which differs from the observed one in CD. The fully automated methodology established by the authors has the potential to be used in

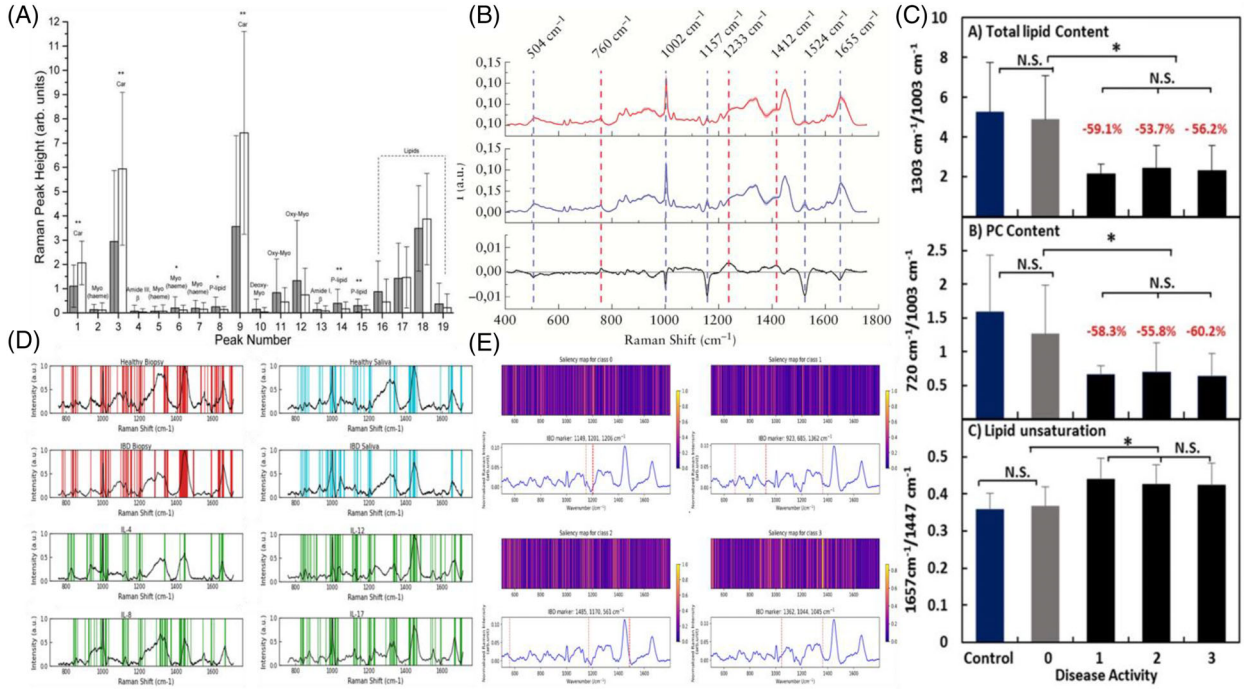

FIGURE 3 IBD discrimination via: (A) average peak intensities for areas of endoscopic inflammation or endoscopic healing, with highlighted peaks of interest indicating standard deviations and *p*-values. ($p^* \geq 0.10$; $p > 0.05$ and $p^{**} \leq 0.05$).[61] (B) Average Raman spectra obtained from patients with CD and healthy controls. Top spectra (red) indicate CD and middle spectra (blue) healthy controls with bottom spectra (black) illustrating the spectral differences between the two sample groups. Dotted lines highlight significant areas of difference between the sample types.[9] (C) Differences in total lipid counts and phosphatidylcholines (PCs) relative to proteins as determined by peak height ratios for differing disease severities.[66] (D) Barcodes derived from tissue biopsy, saliva and potential IBD biomarkers (e) IL-4, (f) IL-12 (g) IL-8 and (h) IL-17, highlighting the dominant, statistically significant peaks. (E) Saliency map visualisations (top) and mean Raman fingerprint regions corresponding to Mayo endoscopy score. A high saliency score on the map is indicated in yellow and represents Raman regions with important contributions in the determination of score classification. Most regions are attributed to proteins, cholesterol and DNA.[36] Reprinted with permission from [9, 36, 61 and 66].

vivo. It also paves the way for further investigation using unclassified IBD samples and, perhaps, patient samples of varying inflammatory status.

Pence et al. exploited the lack of a gold standard in discriminating UC from CD and developed a novel colonoscopy-coupled fibre optic Raman probe as a minimally invasive diagnostic tool. The authors examined spectral signatures from UC, CD and HC patient tissue samples. All samples were compared with tissue pathology markers and assessed using endoscopic examination to confirm the IBD subtype and severity.[12] To separate spectra into their respective groups, a sparse multinomial logistic regression (SMLR) algorithm was applied with variable classification performance of 62–86.2% for sensitivity and of 22.9–74.5% for specificity, with the most successful comparison for control versus active IBD tissue types. A poorer classification performance was observed when comparing healthy controls and inactive IBD subtypes, indicating active inflammation playing a key role. The study built on previous work where it was recognised that increased contact pressure can lead to spectral differences, with attempts made to reduce this effect.[88] Overall, the authors demonstrated that patients with inactive disease exhibited significantly stronger spectra, particularly in bands associated with lipid features. Active disease spectra indicated the broadening of peaks, often associated with an increase in protein content, and possibly originating from increased levels of fibrin and collagen in the bowel wall. The authors also noted the potential impact of age, gender, BMI, diet and previous therapeutic treatments, which most likely impact the classification algorithm. Also, as the probe was in direct contact with the colon, blood and mucus were still present, thus, leading to bias in the obtained spectra.

Furthermore, Acri *et* al. proposed a variation in previous methodology to diagnose and differentiate IBD in paediatric patients. Here, faecal samples were examined to gain insights into the pathological hallmarks of IBD. Of a particular interest was the Amide I protein region, indicating protein conformational and structural changes.[65] The authors applied statistical chemometrics with peak fitting analysis and identified increased spectral intensity at Raman shifts of 1000 and 1300–1800 cm^{-1} (Supporting Information Table S1) when active inflammation is present

and increasing in severity. In terms of protein structure, it was recognised that the presence of active inflammation led to a significant increase in the identified cross-linking ratio between non-reducible and reducible structures, with the UC leading to the most significant increase. From the IBD group, the levels of inflammation were found to vary significantly (161–2360 mg/kg), however, the study did not include further breakdown of patient inflammatory levels, that is, the presence of mild, moderate or severe inflammation. The applied method does, however, demonstrate significant advantages in terms of non-invasiveness, experimental repeatability, low-cost and sample throughput.

Several studies aimed to diagnose either CD or UC, with Veenstra et al. demonstrating one of the first such applications. The authors collected UC and HC tissue samples and examined the mucosal and serosal surfaces, achieving a classification sensitivity of 82% and a specificity of 89% from the mucosal surface and 87% sensitivity and 93% specificity from the serosal surface.[70] Their study indicated the possibility of real-time diagnosis with the ability to observe changes within UC colon tissue which are not apparent in histological examination. Spectral areas of interest revealed significant changes in peaks assigned as proteins, lipids and nucleic acids due to the increase in levels of inflammation. Moreover, Tefas et al. applied a subcategory of RS known as SERS to provide a significantly improved, non-invasive method for UC diagnosis. Plasma samples were collected from UC and HC subjects and analysed using silver nanoparticles as a plasmonic substrate to significantly enhance signal and detection.[67] The primary advantage of SERS over conventional RS is in its ability to offer a significant increase in Raman intensity, leading to the identification of molecules not previously observed in Raman spectra, however, SERS results in a more complex spectral interpretation, particularly, in the case of biological samples, which contain a wide array of metabolites as interferants. Two multivariate classification algorithms were employed for spectral analysis, including PCA-LDA, achieving 86% sensitivity and 92% specificity, and PLS-DA, achieving 89 and 94% sensitivity and specificity, respectively. In UC, there is a known disruption in the gastrointestinal mucosa, which typically maintains the functional status of the bowel.[89] This disruption results in widespread inflammation and, thus, a change in metabolic pathways observed via changes in Raman or SERS spectra, and therefore, enables an insight into the mechanisms of IBD, facilitating the potential development of new diagnostic and therapeutic measures. The major drawback of the study is the overall sample size as well as the exclusion of patients with mild disease. However, it does represent a promising non-invasive approach with the ability to act as a screening tool for colorectal cancer in the longer term.

Similarly, Li et al. demonstrated the non-invasive diagnosis of CD based on SERS combined with a PCA-SVM approach. Urine samples were collected from patients with inactive and active forms of CD as well as HC subjects and combined with silver nanoparticles to form a SERS substrate for analysis. Both PCA-SVM and PCA-LDA were employed to establish classification models to distinguish between CD and HC sample types.[45] Their PCA-SVM algorithm reached a higher classification accuracy than PCA-LDA of 82.5 versus 69.9% between CD and HC groups, respectively. In terms of disease severity, the classification accuracy was higher when comparing active CD/HC subjects than inactive CD/HC subjects at 86.8 and 76.5%, respectively. These findings indicate the potential of RS to effectively identify metabolic changes in patients' urine and to monitor the progress and recurrence of disease.

Morasso et al. applied a similar approach to classification through a combination of RS and PCA-LDA in the diagnosis of CD. Dry plasma samples from CD and HC subjects were analysed with spectral differences between the two groups determined as well as those differences between CD patients with differing disease pattern.[9] The developed PCA-LDA model classified CD and HC subjects with a sensitivity of 80% and a specificity of 85.7%. Biochemical variations were identified between the CD and HC sample types, with the primary difference arising in the carotenoids, compared to the HC and CD spectra, which presented less intense peaks. Further differences were found with respect to the β-sheet secondary structure of proteins and between the lipid and aromatic amino acid bands. This data combined, indicates the systemic metabolic alterations present in patients with CD, where, for instance, the altered levels of lipids could indicate malnutrition due to reduced food intake to alleviate abdominal discomfort or due to the decreased absorptive surface of the bowel. Moreover, the value of this study lies in its ability to find a use as a screening tool in the diagnosis, or exclusion of CD. At present, no accurate blood biomarkers of IBD are available, and those described markers, including ASCA and perinuclear anti-neutrophil cytoplasmic antibodies (pANCAs), suffer from poor sensitivities and, therefore, many patients undergo invasive colonoscopy procedures to rule out CD. A multivariate analysis of blood plasma could potentially present an exciting advance in the development of an effective first-line screening tool for the discrimination of CD.

5.2 | Raman spectroscopic assessment of IBD severity

Assessing disease severity in IBD is crucial for the evaluation of patient care and response to treatment. Due to the

relapsing behaviour of IBD, it is essential that an optimal treatment strategy is chosen and routinely evaluated. Clinically, the assessment of disease severity is validated using symptom scores such as Mayo endoscopic score (MES), Robarts histological index and ulcerative colitis endoscopic index of severity score. However, these scoring systems are often subject to bias, inter-observer variability and the heterogeneity in patient clinical presentation.[36,90,91] Conventional IBD treatments typically result in non-specific inhibition of inflammation, which leads to a reduction in clinical symptoms. Consequently, treatment endpoints have historically centred in assessing the severity of symptoms, despite their limited correlation with mucosal inflammation or the overall impact of the disease-related morbidity and mortality.[92] To this end, it is essential that alternative methods for histopathological diagnosis of disease severity and activity are developed to shorten the time taken to make an informed diagnosis. Such methods, capable of real-time assessment of IBD severity will have inherent advantages, including for instance, monitoring the progression of disease to determine whether current treatments are effective and/or predicting the outcomes of the disease, that is, as an indicator of long-term outcomes. Further, since severe or poorly controlled IBD may lead to complications, including fistulas and structures, early recognition of severe disease state has the ability to prompt interventions to mitigate such risks.[93] It would also lead to improving the quality of life of the patients. Given that the more severe form of IBD often leads to much more pronounced symptoms and, thus, limiting the daily activities to a greater degree, tailored treatment plans based on disease severity would also improve the overall quality of life. Further advantages of direct disease assessment include education—where the patients make a more informed decision related to their care and lifestyle—and healthcare costs, with severe IBD associated with more frequent hospitalisations, surgeries and expensive medicines—where the accurate severity assessments would optimise the more cost-effective strategies.[94,95]

A study focussed on the determination of disease severity by Chernavskaia et al. combines multimodal imaging techniques, including RS, to allow the real-time evaluation of microscopic IBD activity.[62] Both UC and CD tissue samples were examined, with analysis illustrating major indicators of inflammation including crypt distortion, lymphocytic filtrates, rupture of the epithelial barrier and thickening of the basement membrane with primary changes in lipids and proteins. The authors indicate that their findings allow for accurate predictive modelling of the histological index levels, therefore, addressing issues arising from variations in examiners' levels of experience, and ultimately improving the diagnosis and treatment of IBD. This approach could be applied not only after a biopsy for immediate diagnosis, but also, integrated into clinical endoscopy once miniaturised non-linear multimodal probes become accessible. Moreover, Ding et al. studied the in-vivo analysis of mucosal lipids to determine histological disease activity in UC by way of an endoscope-coupled RS. The researchers systematically investigated lipids in inflamed colon tissue, which was correlated with histological assessment of inflammatory status at the same location, and observed that inflamed colon tissue (histology grade 1–3) presented a substantial decrease (50–60%) in phosphatidylcholine content and total lipid count when compared to quiescent and control tissue (grade 0), indicating that active inflammation reduces the overall level of lipids in the colonic mucosa.[66] However, for the lipid count comparing control and grade 0 tissue, no significant differences were observed, and similarly, between the three inflammatory grades (1–3), thus indicating, lipid content could serve as a potential spectral marker in the assessment of disease activity. The advantage of the presented technique over current qualitative methods, for example, mass spectrometry, is in its suitability and ease of use for the in-situ analysis of the colonic mucosa during endoscopy, eliminating the need for invasive biopsies. A further study with a similar focus in the field of RS assessment of IBD disease severity by Kirchberger-Tolstik et al. correlates Raman spectra obtained from tissue with the four-level Mayo score used to indicate endoscopic disease severity. A one-dimensional deep convolution neural-network (1D-CNN) was applied to produce a predictive model of the Raman spectroscopic data,[36] achieving a sensitivity and specificity of 78 and 93%, respectively. The main observable differences between spectra from normal and inflamed tissue indicate the presence of increased levels of proteins, lipids and DNA. The authors demonstrate the ability of the methodology to classify fresh biopsy samples as well as diseases beyond the IBD and describe a low-risk diagnostic procedure with a real-time to results workflow.

Wu et al. used SERS to detect changes in the urine of patients with active and inactive forms of CD. Applying PCA-SVM to the datasets, an accuracy of 75.5% was obtained. When identifying disease location, either colonic or ileal-type tissue, the authors observed a significant increase in the 1643 cm^{-1} Raman band assigned to the C-C stretch in lipids and the C-O stretch of protein amide I band.[71] Interestingly, spectral changes associated with treatment of tumour necrosis factor inhibitor were also investigated in this study, where the classification accuracy of before and after treatment groups achieved 91.2%. Again, most of the metabolic changes in urine samples were attributed to proteins, lipids and amino acids. This study indicates the potential of RS in assessing the severity of IBD as well as monitoring patient responses to treatments. An associated limitation of the study stems from the

exclusion of healthy control samples with a larger cohort required to provide validation for the suitability of the methodology in both assessing disease severity and patient response to therapeutics.

5.3 | Mucosal healing in IBD

Endoscopic MH is defined as 'complete absence of all inflammatory and ulcerative lesions' upon endoscopy.[92] In recent years, MH has been proposed as a superior measure compared to clinical symptoms in predicting the effectiveness of treatments as well as disease trajectory. The primary objective of treatment for IBD patients has traditionally been to induce and maintain symptomatic improvement, or ideally, to achieve remission, leading to improved quality of life for patients. However, due to varying interpretations of MH, challenges associated with reproducibility of IBD scores among different observes and potential microscopic inflammation in mucosa which appears healthy during endoscopy, there is an urgent need for enhanced techniques to aid in defining treatment endpoints for IBD.[96]

Raman spectroscopic analysis has been in recent years proposed as a suitable methodology in differentiating mucosal healing from inflamed tissue. The first study published on this topic by Addis et al., investigated colon biopsy samples from quiescent (mucosally healed) and inflamed tissue in patients with UC. The suitability of RS in discriminating tissue, which has been described as histologically healed and tissue for which histological inflammation is still present, was assessed.[61] The authors identified clear differences between the two tissue types with significant changes observed between the carotenoid peaks and two different phospholipid peaks.[61] These changes were attributed to the role of carotenoids as a defence mechanism against inflammation and that during inflammation, tissue loses integrity due to ulceration with such disruption leading to an expected decrease in phospholipids, further consistent with damage to the cell membrane. This study was limited to the single scan measurements of tissue biopsies and endoscopic scoring using the MES as opposed to the recently developed Paddington international virtual ChromoendoScopy ScOre (PICaSSO), which correlates strongly with the five most commonly applied histological indices in predicting histological remission.[91]

Smith et al. built upon this and further identified spectral changes before and after treatment, as well as the ability of RS to differentiate between MH and inflammation combined with rapid machine learning classification. The authors identified a significant decrease in peak intensity at Raman bands of 1003 and 1252 cm^{-1} post-treatment, and also when MH was observed.[64] Of further interest was the increase in intensity at 1304 cm^{-1} detected in MH.[82] The employed SKiNET algorithm achieved sensitivity and specificity of 96.3 and 95% in UC and 96.2 and 88% in CD patients, respectively. The detected spectral intensity increases were attributed to the inflammation present in both UC and CD tissue samples, with phenylalanine associated with immune activation and an influx of inflammatory cells, similar to the previous studies, attributed to phospholipids with disruption in the bowel wall of inflamed tissue. Since this study was conducted ex-vivo, optimisation of laser power and exposure times would be a primary requirement to enable translation into in-vivo clinical translations.

5.4 | Biomarkers in IBD via RS

Diagnosis, evaluation of severity and prognosis still pose challenges for physicians in the clinic. The identification of potential biomarkers in IBD would, therefore, offer important advantages, including for instance, a more objective assessment, thus, reducing the reliance on subjective evaluation of symptoms as well as early diagnosis of disease, therefore, allowing timely interventions and improved outcomes. These would also offer treatment guidance in tailoring therapeutic strategies, monitoring of disease progression and a reduction in invasive procedures. Their use can, therefore, lead to an overall more effective and efficient healthcare delivery with improved outcomes for patients with IBD.[97,98]

Currently, blood and stool-based biomarkers offer reproducible and quantifiable resources which aid clinicians in the diagnosis and management of IBD with C-reactive (CRP), faecal calprotectin (FC) and lactoferrin being the only biomarkers routinely used in clinical practice.[99–101] CRP is an objective marker of inflammation in the assessment of disease activity, however, it correlates less well in patients with UC compared to CD. It also has a lower sensitivity for mild or localised disease and as such, is mostly limited to more moderate or severe inflammation. In addition, while faecal markers, such as FC and lactoferrin, appear promising and may be more specific in the detection of gut inflammation, these markers require stool collection, often perceived as unpleasant for the patient. Recent studies also indicate the superior performance of FC in the detection of UC compared to CD. Further possible blood biomarkers of IBD have been described in the literature with conflicting results. Perhaps the most well-known biomarkers are ASCA and pANCAs, however, their sensitivity is currently poor at only 55%,[102] thus, defeating their purpose.

An optimal IBD biomarker detection technique should be simple, straightforward, minimally invasive, cost-effective and rapid. It must also be reproducible between patients and laboratories and should possess predictive significance concerning the likelihood of disease relapse of recurrence. In pursuit of this objective, RS could aid in IBD biomarker identification via the enhanced understanding of the molecular basis of IBDs and combined with machine learning, would uncover complex relationships and patterns within large datasets, leading to the discovery of new candidate biomarkers. A recent study by Buchan et al. demonstrated RS for methodical profiling and classification of potential IBD-indicative biomarkers towards establishing important biochemical 'fingerprints' as characteristic barcodes for on-going and future studies of diagnostic and prognostic applications in IBD with the acquired spectral data classified using an ANN algorithm, SKiNET as a decision support tool. The authors identified four specific cytokines that were either over- or -underproduced, thus, offering valuable insights regarding the fundamental mechanism of disease. Cytokines IL-4, IL-8, IL-12 and IL-17 were found to exhibit specific Raman bands characteristic of patients with IBD of 936, 1003, 1340, 1445 and 1656 cm^{-1}. Cytokines are known to play a central role in the modulation of the intestinal immune system. Produced predominantly by lymphocytes and macrophages, cytokines have either pro-inflammatory functions (IL-4) or anti-inflammatory functions (IL-8 and IL-12). Previous studies using techniques, such as enzyme-linked immunosorbent assay, have indicated differing levels of mucosal and systemic concentrations of pro- and anti-inflammatory cytokines in patients with IBD, and here the ability of RS as a non-invasive technique for the multiplexed profiling of saliva and tissue biopsies has successfully established unique molecular barcodes for candidate biomarkers of IBD.

Overall, laboratory biomarkers are valuable and should be integrated into the comprehensive management of IBD. Nonetheless, it's essential to recognise they are not a panacea, and until additional data becomes accessible, the use of CRP and other laboratory markers should be regarded as a supplementary aid to clinical observation and physical examination rather than a substitute. Building upon demonstrating relevant RS applications for the clinical and biomedical fields, including biomarker discovery and patient stratification, with the potential to contribute significantly to the development of new diagnostic methodologies and therapeutic monitoring, further studies validating these findings will lay the platform for defining functional relevance in the complex aetiology of IBD, as well as cementing RS as a powerful technique for discovery of biomarkers in other diseases and biological samples with many ramifications.

6 | CONCLUSIONS AND OUTLOOK

This comprehensive review highlights the significant strides made in exploiting the potential of RS as an emerging diagnostic tool in IBDs. The breadth of emerging studies, collectively, emphasise the promise of RS in providing crucial understanding along with timely detection of the molecular and structural changes occurring in IBD-affected tissues such as the colon. RS, by providing unique biomolecular spectral fingerprints of target analytes with a very rapid analytical response, enabling non-destructive, label-free, quantitative analysis of composition and structure with an inherently straightforward detection and no complex sample preparation, renders itself as a powerful in-situ technique, yielding quantitative outputs. The non-invasive nature combined with the ability to offer real-time rapid analysis has been cementing RS recognition as a valuable adjunct to conventional diagnostic methods.

It is worth noting that, albeit significant progress has been made in these fields, challenges remain. One potential issue for real-world adoption is a lack of standardisation in the methods used within the current literature, from matters pertaining to biofluid collection, storage and pre-processing, to those concerning Raman measurement protocols and chemometric analyses and classification. Standardisation of protocols and conducting larger-scale clinical studies are vital for advancing the clinical translation of RS in IBD diagnostic and prognostic applications. While RS has shown promise in research settings, there is limited clinical evidence demonstrating its diagnostic accuracy and clinical utility, with many presented studies comprising small sample sizes. Thus, extensive clinical evaluation with much larger sample cohorts is an essential requirement in the clinical translation of RS-IBD. A further challenge associated with RS transition from benchtop to clinical laboratory and the bedside, is the complexity and cost of instrumentation. RS systems currently, require specialised personnel to operate, are often expensive and cumbersome to maintain. However, with the development and advancement of portable Raman instrumentation, many of the existing limitations, such as user-friendly interface, flexibility and point-of-care testing, are concurrently being addressed, rendering it an increasingly valuable tool in healthcare settings. RS, further combined with advanced machine learning techniques, enables the enhancement of the accuracy, reliability and speed of IBD diagnosis and the imminent automated classification of Raman spectra along with assignment to a particular IBD

biomarker, tissue type or disease state is perhaps the most important step for the translation of Raman based diagnostics to real world, clinical applications. Moreover, hybrid RS-AI methodologies have the ability to support large-scale screening for IBD risk factors or early signs of the disease, particularly useful for identifying high-risk populations and implementing preventative measures given both the increased incidence and the prevalence of IBDs.

Overall, the expanding interdisciplinary collaborations between clinicians, scientists and engineers, which are pivotal to unlocking the full potential of RS for IBDs, are starting to bridge the gap between clinical research and practice, with the emerging findings being translated into real-life benefits for patients including, timely IBD diagnosis, improved disease management and continued bedside monitoring. With the ability of RS to distinguish different diseased states, determine therapeutic response to treatment as well as characterise the complex microenvironment of the gut, this spectroscopic technique holds a further great potential to transform the early-stage IBD diagnosis and the long-term monitoring.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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