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Non-invasive cholesterol assessment: current methods and future perspectives

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Cholesterol plays a vital role in human physiology. However, elevated levels of cholesterol in the blood affect the functioning of vital organs that are prone to various diseases. Therefore, identifying cholesterol and maintaining it beyond the threshold values is required. However, traditional invasive methods are cumbersome and potentially harmful. This review focuses on non-invasive cholesterol assessment, with particular emphasis on optical spectroscopy and imaging techniques that enable cholesterol estimation without biofluid extraction. This review examines the recent advancements in optical and non-optical cholesterol monitoring techniques, highlighting their strengths and limitations. Non-optical approaches offer cost-effective solutions for point-of-care testing, while considering the sensitivity and real-time capabilities, optical methods like Raman and Infrared spectroscopy have demonstrated significant advancements. Despite these advancements, challenges remain in accuracy, biological variability, and user accessibility, which are some of the constraints noted in different optical approaches. Developing robust, reliable measuring systems through ongoing research is essential for disease detection due to cholesterol. This paper aims to guide prospective research efforts towards innovative portable devices for effective cholesterol management.

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1. Introduction

The World Health Organization (WHO) has emphasized the severe health repercussions of excessive cholesterol. High cholesterol levels have been reported to result in nearly 2.6 million deaths annually (WHO, 2021, 4th edition).[1] High cholesterol is a key risk factor for various life-threatening disorders, including cardiovascular diseases (CVDs), which are the leading cause of mortality worldwide, accounting for approximately 18 million deaths each year.[2] Elevated cholesterol levels are a major risk factor for various health conditions, including cardiovascular disease and stroke, resulting in approximately 4.4 million deaths worldwide each year.[3] Research on cholesterol has been a major focus of scientific study during the 20th century.[4] Cholesterol and glucose are two essential substances of the human body, with glucose and fatty acids being the primary sources of energy.[5] Cholesterol plays a crucial role in the functioning of various organs,[6] as it forms a critical part of cell membranes, aids in the synthesis of steroid hormones through its involvement in remnant lipoprotein particles,[7,8] and is necessary for the development and maintenance of fetal tissues.[9,10] Additionally, cholesterol acts as a hepatic regulator and dietary constituent.[11,12] Cholesterol is a vital substance in the human cell membrane, where it plays an important role in the formation of bile acids in the liver,[13,14] and in the synthesis of vitamin D[15] and steroid hormones.[16] Cholesterol in humans has been identified in two forms: approximately 30% as sterol and 70% as esterified with fatty acids.[17] Understanding these forms of cholesterol is important for recognizing its significance in health and disease.

The chemical formula for cholesterol is $C_{27}H_{46}O$[18] and its structure is mentioned in the Fig. 1.

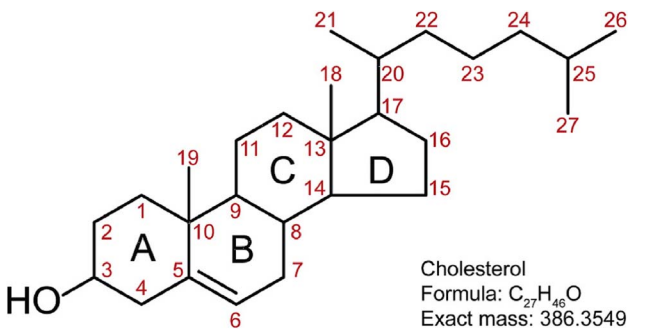

Fig. 1 [18]Chemical structure of cholesterol showing the arrangement of rings, hydrocarbon chain, and hydroxyl group.

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Table 1 Clinical classification of low-density lipoprotein (LDL) cholesterol levels and associated cardiovascular risk categories

LDL cholesterol level (mg dL^{-1})	Category
Less than 100	Excellent
100–129	Good
130–159	Moderate
160–189	Unhealthy
190 and above	Critical

In 1769, Poulletier de la Salle first discovered cholesterol in the solid form within bile and gallstones.[19] Cholesterol is transported through the bloodstream by molecules known as lipoproteins,[20] which are a combination of fat (lipids) and proteins. Lipoproteins can be categorized into three types.[21] LDL (Low-Density Lipoprotein) – This is referred to as harmful cholesterol. It transports cholesterol from the liver to different cells in the body.[22] LDL can accumulate in artery walls and form plaque. This plaque buildup can narrow the arteries, restricting blood flow and increasing the risk of heart attack and stroke. The characteristics and classification of various levels of LDL cholesterol have been thoroughly described in Table 1.

• High-density lipoprotein (HDL) is often known as good cholesterol, and it helps transport excess cholesterol from cells to the liver,[23] where it can be processed and removed from the body, thereby preventing the buildup of plaque in arteries. A thorough description of the features and categorization of different HDL cholesterol levels has been provided in Table 2.

• Triglycerides (TG) – Dietary triglycerides are the primary form of fat. Unused food is converted into triglycerides and stored in fat cells. Hormones like cortisol and adrenaline play a crucial role in regulating the body's metabolism, including the breakdown and usage of stored triglycerides (fats).[24,25] A high triglyceride level, along with low HDL cholesterol or high LDL cholesterol, is associated with the formation of fatty deposits in artery walls, which raises the risk of heart attack, peripheral arterial disease, and stroke. Table 3 shows various Triglyceride levels and their nature.

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Table 2 Clinical classification of high-density lipoprotein (HDL) cholesterol levels and associated cardiovascular health categories

HDL cholesterol level (mg dL^{-1})	Category
60 and above	Excellent
35–45	Moderate
Less than 35	Unhealthy

One of the primary conditions associated with high cholesterol is atherosclerosis,[26] where cholesterol deposits form plaques in the arteries, causing them to narrow and harden, which can result in heart attacks, strokes, and PAD.[27] Peripheral Artery Disease (PAD) occurs when plaque, which is primarily composed of cholesterol, builds up in the arteries of the limbs, leading to decreased blood flow and symptoms such as discomfort, numbness, or pain, and, in severe cases, tissue death.[28] Elevated cholesterol levels are associated with an increased risk of several severe illnesses, including red blood cell deficiency, overactive thyroid, nutrient malabsorption, high blood pressure, cardiovascular disease, coronary heart disease, and brain clots. To prevent these conditions and maintain normal health, it is essential to regularly monitor cholesterol levels in the blood. Studies have consistently found a solid link between high cholesterol levels and an increased risk of cardiovascular events. For example, consider the Framingham Heart Study,[29] a long-term investigation of cardiovascular risk factors. Given the major consequences of elevated cholesterol, proper control and monitoring are required. Regular cholesterol screenings are essential for identifying individuals at high risk of various heart diseases and enabling early intervention. Individuals may drastically reduce their chance of acquiring high cholesterol and its related consequences by adopting good lifestyle behaviors such as eating a balanced diet, exercising regularly, and avoiding tobacco use.

In this review, non-invasive cholesterol detection is strictly defined as methods that do not require blood or other biofluid extraction. Accordingly, optical spectroscopy and imaging-based techniques constitute the core of truly non-invasive cholesterol assessment, while electrochemical and biosensor-based approaches are classified as minimally invasive or indirect methods. In recent years, there has been a renewed and accelerated research focus on non-invasive and wearable chemical sensing technologies for metabolic health monitoring. Advances in materials science, signal processing, and miniaturized electronics have enabled on-body platforms capable of detecting biomarkers from alternative biofluids such as sweat, saliva, tears, and breath.[30,31] Among the optical detection modalities evaluated in this review, photoacoustic spectroscopy (PAS)[32] has emerged as a particularly promising non-invasive approach, combining pulsed optical excitation with ultrasonic detection to achieve deeper tissue penetration and molecular-level contrast than purely optical techniques, making it well-suited to cholesterol-related lipid characterisation in subsurface tissue layers. The study is structured to provide a comprehensive overview of cholesterol monitoring approaches as follows: Section I highlights the significance of cholesterol in human health. Section II demonstrates the materials and methods used for the review process. Section III categorizes the monitoring techniques into optical and non-optical, detailing their principles and clinical applications. Section IV focuses on non-invasive cholesterol detection using non-optical and optical techniques, including their benefits, limitations, and latest research findings. Section V provides a comparative analysis of these techniques. Section VI focuses on Wearable sensors and Global applicability of POC diagnostics. Section VII addresses the challenges and future directions in cholesterol monitoring and Section VIII concludes by emphasizing the need for reliable cholesterol monitoring systems.

Table 3 Clinical classification of triglyceride levels and associated metabolic and cardiovascular risk categories

Triglyceride level (mg dL^{-1})	Category
Less than 150	Regular
150–199	Moderate
200–499	Unhealthy
500 and above	Critical

2. Materials and methods

This work adopts a narrative review methodology, focusing on comparative and technological analysis rather than a formal systematic review.

2.1. Literature review and selection criteria

A systematic approach was adopted to identify and select relevant literature for this review. The methodology is as follows:

• *Database selection*: the literature has been conducted using major academic databases, including PubMed, IEEE Xplore, Web of Science, and Scopus, due to their comprehensive coverage of biomedical and engineering research.

• *Search strategy*: keywords such as "non-invasive cholesterol detection," "optical methods for cholesterol monitoring," "non-optical approaches," and "cholesterol assessment techniques" were used in various combinations. This review mainly focused on recent articles published on "non-invasive and optical, non-optical techniques" from 2013 to 2025. Accordingly, the comparative literature summaries have been updated to include studies published up to 2025, and the newly added references are incorporated in Tables 8–13.

• *Screening process*: the literature initially identified a total of 193 articles from various major academic databases. Following the screening of titles and abstracts, approximately 133 articles are identified as more suitable for non-invasive cholesterol detection methods. A comprehensive evaluation of these shortlisted articles was conducted to assess their quality, experimental rigor, and alignment with the review's scope. This evaluation resulted in the inclusion of approximately 60 studies that provided substantial data on either optical or non-optical cholesterol detection techniques. In addition to the core

screened studies, foundational reviews, standards, and recent reports were included to ensure completeness.

2.2. Inclusion criteria

Studies were deemed eligible for inclusion as they met the following criteria:

- Published in peer-reviewed journals or presented in indexed conference proceedings.
- Focused explicitly on the development, optimization, or comparative analysis of non-invasive cholesterol detection methods.
- Provided detailed experimental procedures, validation results, or clinical applications.
- Demonstrated clear alignment with the review's scope, specifically emphasizing optical and non-optical cholesterol detection techniques.

2.3. Exclusion criteria

The following types of studies were excluded:

- Articles not published in English.
- Studies exclusively discussing invasive cholesterol detection techniques.
- Reviews or meta-analyses lacking primary experimental data.
- Publications with insufficient technical details or lacking experimental validation.

2.4. Rationale for depth of discussion

The review encompasses a broad spectrum of cholesterol detection techniques, intentionally structured to provide a comprehensive overview of both well-established and emerging methods. The depth of discussion for each technique was carefully determined based on its maturity, clinical applicability, and the availability of substantial experimental evidence in the literature, as follows

Prioritization of techniques:

- *Established methods*: techniques such as infrared spectroscopy (IR) and Raman spectroscopy were analyzed in greater detail due to their advanced development and demonstrated precision in non-invasive cholesterol detection. For instance, IR spectroscopy's capability to detect specific absorption peaks associated with hydroxyl (–OH) and C–H groups enables accurate cholesterol quantification. Similarly, Raman spectroscopy's ability to differentiate cholesterol deposits in deep tissue has significant implications for diagnostic applications.
- *Emerging methods*: methods like Photoacoustic Spectroscopy (PAS), sweat/tear analysis, and transdermal biosensors were described briefly to highlight their potential while acknowledging their current limitations, such as limited clinical validation and scalability.
- *Critical analysis framework*: a structured comparative framework was employed to evaluate each technique based on:
- *Performance metrics*: sensitivity, specificity, detection limits, and real-time capabilities.
- *Practical challenges*: biological variability, calibration requirements, cost-effectiveness, and technical feasibility.
- *Clinical relevance*: applicability to point-of-care settings and potential for commercialization.

Optical methods, particularly Raman and infrared spectroscopy, were subjected to a detailed analysis due to their molecular specificity and promising results in non-invasive settings. On the other hand, non-optical approaches such as impedance spectroscopy and electrochemical sensors were discussed in terms of their cost-effectiveness and suitability for portable devices, albeit with noted limitations in sensitivity and specificity.

Acknowledgement of limitations: while the review aims for comprehensiveness, variations in the depth of discussion were unavoidable due to differences in the maturity of techniques and the volume of supporting literature. The review recognizes that emerging methods like PAS and microwave techniques warrant deeper analysis as more experimental and clinical data become available. Future work will emphasize enhancing the critical evaluation of these emerging approaches to ensure uniformity in discussion.

2.5. Contribution and future perspectives

While this manuscript primarily reviews existing literature on non-invasive cholesterol detection techniques, it also offers critical comparisons, identifies research gaps, and provides guidance for future investigations. The review evaluates both optical and non-optical methods, highlighting their advantages, limitations, and clinical applicability. For instance, infrared spectroscopy is emphasized for its molecular specificity, while Raman spectroscopy is noted for its potential in deep tissue analysis. Emerging methods like PAS and microwave techniques are acknowledged for their promise but require further validation. The manuscript identifies key research gaps, such as addressing biological variability, improving calibration protocols, and achieving clinically acceptable sensitivity in techniques involving biofluids like saliva and sweat. To guide future research, hybrid approaches are proposed, combining optical and non-optical methods to overcome individual limitations, along with integrating machine learning algorithms to enhance spectral analysis accuracy. The development of wearable biosensors and portable devices for real-time cholesterol monitoring is also highlighted as a critical area for advancing clinical practice. By synthesizing existing knowledge and proposing novel perspectives, this review not only provides a comprehensive summary of the field but also serves as a roadmap for future research on non-invasive cholesterol detection.

3. Cholesterol measuring techniques

The classification of cholesterol measurement techniques is based strictly on the nature of biological sampling rather than on the physical location where testing is performed. Cholesterol measuring techniques are broadly classified into invasive, minimally invasive and non-invasive methods, as shown in Fig. 2.

3.1. Invasive cholesterol detection techniques

Invasive cholesterol measuring techniques require direct access to the vascular system through venous or arterial blood sampling and are predominantly used in clinical laboratory settings.

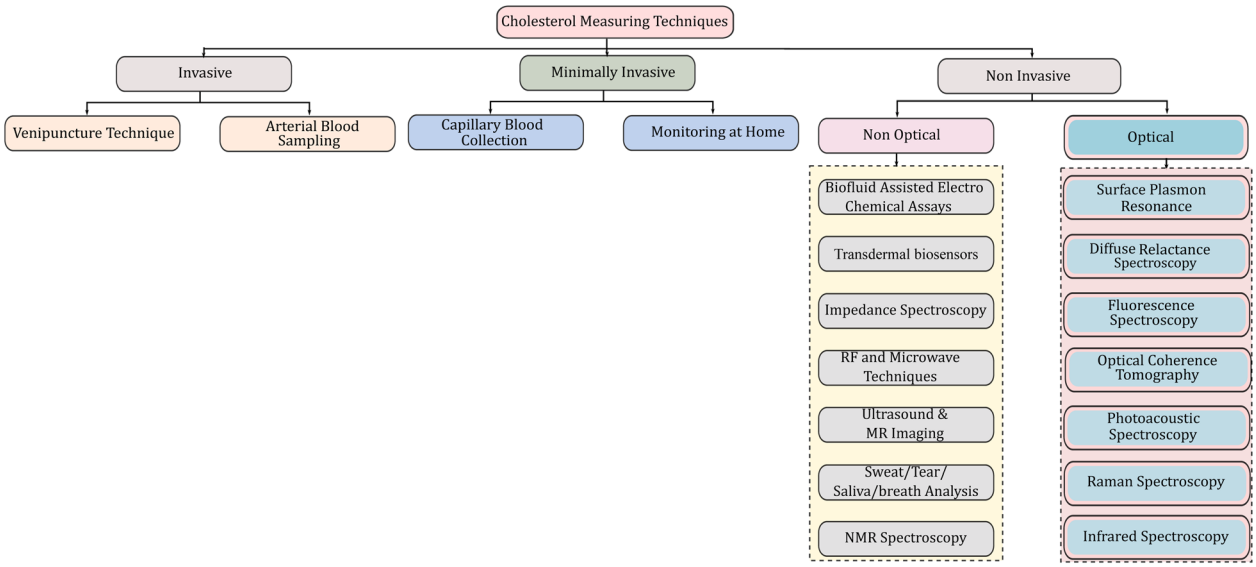

Fig. 2 Classification of cholesterol measurement techniques based on the level of invasiveness and sensing modality.

Traditionally, cholesterol has been assessed by simple chemical tests to complex laboratory procedures.[33] Typically, invasive procedures require drawing blood samples, which are then analyzed using various techniques to measure cholesterol levels. Modern approaches such as enzyme assays, chromatographic procedures, mass spectrometry, and nuclear magnetic resonance have been replaced the conventional methods like the Liebermann–Burchard reaction. Each method offers unique advantages and limitations, making them suitable for various research and clinical applications.[34,35] The quality of the blood sample collection significantly influences the accuracy of cholesterol measurement. There are different techniques for drawing blood samples; each has distinct procedures that can affect cholesterol determination.

3.1.1. Venipuncture technique. The most common technique for drawing blood for cholesterol testing is venipuncture. This involves puncturing a vein, typically in the arm, with a needle to extract blood into a collection tube. This approach is recommended because it can supply enough blood to allow for a thorough lipid profile. Proper handling of the blood sample is crucial to prevent hemolysis, which can interfere with accurate cholesterol measurement,[36].

3.1.2. Arterial blood sampling. Arterial blood sampling provides extremely accurate measurements of blood gases and other analytes. It is typically performed on the radial or femoral artery.[37] Arterial blood sampling is primarily used for measuring blood gases and other analytes, rather than for routine cholesterol testing. However, it can be essential in critical care or research settings where precise data is required. Compared to venipuncture, the procedure is more invasive and has a higher risk of complications.

Several laboratory techniques can be used to assess cholesterol levels after the blood sample is obtained, as shown in Fig. 3.

The concepts mentioned above, working techniques, and performance measurements vary significantly among different laboratory examination techniques. Table 4 provides a comparison of these laboratory examination techniques.

3.2. Minimally invasive cholesterol detection techniques

Minimally invasive cholesterol measuring techniques involve limited disruption of the skin barrier, most commonly through capillary finger-prick blood sampling, and form the basis of home and point-of-care monitoring systems.

3.2.1. Capillary blood collection. A small blood sample is acquired through capillary blood collection by pricking the fingertip or earlobe. This method is more convenient and less invasive than venipuncture, making it suitable for self-monitoring and point-of-care testing.[48] On the other hand, the limited blood volume obtainable may restrict the types of analyses that can be performed, and inconsistencies in sample quality can impact the accuracy of results.

3.2.2. Monitoring at home. Home monitoring of cholesterol offers convenient ways to track cholesterol levels without

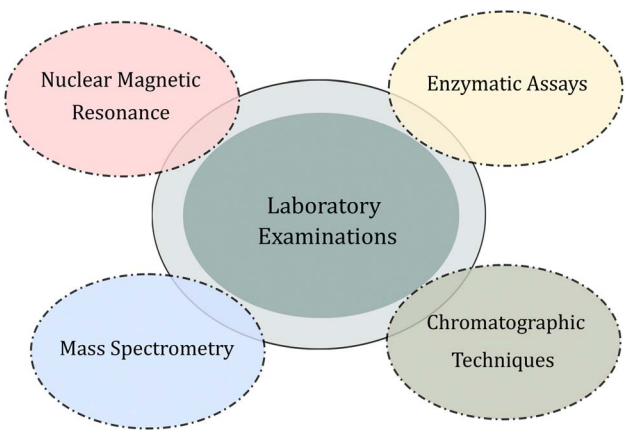

Fig. 3 Conceptual illustration of laboratory examination techniques applied to cholesterol analysis.

Table 4 Comparison of laboratory-based cholesterol measurement techniques highlighting analytical principles, performance characteristics, and clinical applications

Method	Principle	Procedure	Advantages	Limitations	Time required	Cost	Sensitivity	Specificity	Accuracy	Clinical applications	Ref.
Enzymatic assays	Enzymatic color reaction	Serum separation and absorbance measurement	Simple, speed, and economical	Interference from blood constituents	Short	Low	High	Moderate	High	Routine clinical use	38 and 39
Gas chromatography (GC)	Separation *via* gas flow	Lipid extraction derivatization GC detection	Detailed lipid profiling	Extensive preparation, complex instrumentation	Long	High	Very high	Very high	Very high	Research, detailed analysis	40 and 41
High performance liquid chromatography (HPLC)	Liquid phase separation	Lipid extraction and HPLC/LC-MS detection	Shorter analysis time	Requires sophisticated instrumentation and skilled operators	Moderate	Moderate	High	High	High	Clinical and research	42 and 43
Mass spectrometry (MS)	Ionization and mass analysis	Ionization, ion separation mass analysis	Gold standard for lipid analysis	Advanced instrumentation and expertise required	Long	Very high	Very high	Very high	Very high	Research, detailed analysis	44 and 45
Nuclear magnetic resonance (NMR)	Magnetic properties of nuclei	Sample dissolution and spectral analysis	Non destructive structural information	Expensive instrumentation, and specialized expertise	Long	High	High	High	High	Research, specialized use	46 and 47

Table 5 Overview of home-based cholesterol monitoring methods focusing on key features, advantages, and limitations

Method	Key features	Merits	Demerits	Source
Home cholesterol test kits	Disposable test strips, lancets, small reader	Simple, convenient, quick results	User error, accuracy can vary	AmeriHealth Caritas
Cholesterol monitors	Digital display, memory function, bluetooth connectivity	Comprehensive results, data storage, easy tracking	Higher cost, requires more initial investment	WebMD
Mail in cholesterol test kits	Sample collection materials, pre paid mailing envelope	Lab quality results, no need for clinic visits	Longer turnaround time, potential shipping issues	NCBI
Smartphone connected devices	Wireless connectivity, smart phone apps	Data integration, easy sharing comprehensive tracking	Expensive, requires compatible smart phone	Mayo Clinic
Continuous monitoring devices	Wearable sensors, real time data transmission	Real time monitoring, early detection	Not widely available, accuracy challenges	AHA journals

frequent doctor visits. These methods vary from simple test kits to more sophisticated portable monitors. Table 5 summarizes various methods of measuring cholesterol at home.

One of the limitations of home cholesterol monitoring is the potential for erroneous results, leading to difficulties in interpreting the data. Additionally, users may experience psychological strain and face increased expenses for supplies and equipment. For the appropriate use of machines, effective monitoring requires careful consideration of costs and resource allocation. Table 6 lists commercial cholesterol monitoring devices used for home monitoring.

As technology advances and home monitoring devices integrate with telehealth and electronic health data, they will become an essential tool in modern healthcare.[56]

4. Non-invasive cholesterol detection techniques

Non-invasive cholesterol measuring techniques estimate cholesterol levels without blood extraction or skin penetration, relying instead on external sensing modalities or alternative biofluids. Non-invasive cholesterol detection methods are favoured over invasive techniques for a variety of reasons. Painless needle-free techniques are helpful for the regular monitoring of cholesterol, which enhances the outcomes of the patient. Moreover, these methods significantly reduce the risk of infection associated with intrusive procedures, ensuring a safer experience. By avoiding blood draws, laboratory processing, and medical waste disposal, non-invasive testing also leads to substantial cost savings. Overall, non-invasive cholesterol testing procedures prioritize the patient's well-being, convenience, and economic efficiency. Non-invasive cholesterol detection techniques are categorized into two approaches non-optical and optical.

4.1. Non-optical techniques

These techniques rely on principles other than light interaction for cholesterol detection. There are various non-optical techniques used for cholesterol detection.

4.1.1. Biofluid-assisted electrochemical assays. Electrochemical sensors function by the measurement of the electrical reaction that arises from a chemical reaction. These sensors have the advantage of being highly sensitive, particular, and able to reduce size for portability. The two main categories of electrochemical sensors are enzyme-based and non-enzymatic sensors.

- *Enzyme-based electrochemical sensors*: enzyme-based sensors utilize cholesterol oxidase or cholesterol esterase to catalyze processes that produce observable signals. For instance, Lee *et al.* (2018) developed an enzyme-loaded paper-based impedimetric sensor for non-invasive saliva cholesterol detection.[57]

A recent critical review highlights that nanostructured metal oxides, carbon-based composites, and hybrid electrode architectures offer improved operational stability and reduced interference effects compared to enzyme-based platforms.[58]

- *Non-enzymatic electrochemical sensors*: non-enzymatic sensors accelerate cholesterol oxidation using metal oxides or nanoparticles without biological enzymes. For instance, Benny *et al.* (2021) reported a sensitive and eco-friendly sensor using Fe_2O_3 composite electrodes and piper nigrum-derived porous carbon.[59] Metal nanoparticles are employed as electrode materials due to their high conductivity and catalytic activity. For instance, Wang *et al.* (2021) utilized nanoporous gold for precise cholesterol detection.[60] Carbon-based materials like graphene and carbon nanotubes offer large surface areas and superior conductivity. For instance, Geetha *et al.* (2023) demonstrated a sensor using graphene oxide composites.[61]

To expand the clinical utility of these sensors, future research should prioritize enhancing selectivity, stability, and user-friendliness.

4.1.2. Transdermal biosensors. Transdermal biosensors offer a promising non-invasive approach for continuous monitoring of various biomarkers, eliminating the need for blood sample collection. These sensors can be categorized into micro needle-based and non-micro needle-based types.

- *Micro needle-based sensors*: microneedle sensors minimally penetrate the skin to access interstitial fluid, which reflects blood cholesterol levels. Liu *et al.* (2020) gave a summary of transdermal biosensors based on micro needles and highlighted their potential for the detection of cholesterol and glucose.[62] These minimally invasive technologies provide high sensitivity and specificity.

Table 6 Assessment of approved cholesterol-related monitoring products based on measurement technique and real-world applicability

Apparatus	Firm	Primary output	Technique	Target point	Direct cholesterol measurement	Correlation with gold-standard venous enzymatic lipid panel	Real-world reproducibility and applicability	Authority	Ref.
CardioChek	PTS diagnostics	TC/HDL/TG	Handheld analyzer	Finger stick	Yes	TC: $r = 0.67$; HDL: $r = 0.64$; TG: $r = 0.91$; LDL: $r = 0.75$	Sensitive to operator handling, strip/lot variation, hematocrit and storage; periodic lab cross-check recommended	FDA cleared	49
Cholestech LDX	Abbott	TC/HDL/TG	POC enzymatic cassette analyzer	Finger stick	Yes	TC: $r = 0.96$; HDL: $r = 0.88$; LDL: $r = 0.87$; TG: $r = 0.99$	Requires standard handling; field performance depends on user training and cartridge storage	FDA cleared	50
Philips Lumify	Philips	Carotid imaging metrics	Ultrasound	Carotid artery	No	NA	Protocol dependent; useful for vascular risk assessment	FDA cleared	51
LipoScience NMR LipoProfile	LabCorp	Lipoprotein particle profile + lipid parameters	NMR spectroscopy	Venous blood	Yes	TC: $r = 0.964$; HDL: $r = 0.83$; LDL: $r = 0.94$; TG: $r = 0.97$	High laboratory reproducibility; requires venipuncture and laboratory infrastructure	FDA cleared	52
Cardea SOLO	Cardiac insight	ECG rhythm	Electrocardiogram	Chest	No	NA	Performance depends on wear time, skin contact and motion artefacts; adjunct cardiovascular monitoring tool	FDA cleared	53
Eko DUO	Eko health	ECG + digital auscultation	Electronic stethoscope + ECG	Chest	No	NA	Dependent on acquisition conditions, adjunct cardiac assessment tool	FDA cleared	54
FibriCheck	FibriCheck	PPG-based rhythm	Smartphone camera + AI	Finger	No	NA	User- and motion-dependent; validated for rhythm screening	CE marked	55

FDA cleared:- approved by the United States Food and Drug Administration for clinical use; CE marked:- compliant with European Union medical device regulatory requirements.

• *Non-micro needle-based sensors*: non-micro needle techniques for obtaining interstitial fluid often necessitate more invasive procedures. Madden *et al.* (2020) talked about the use of micro needle-based electrochemical devices for bio-sensing in dermal interstitial fluid to detect cholesterol, uric acid, and glucose simultaneously.[63] Materials like carbon nanotubes and graphene enhance sensor sensitivity due to their large surface area and excellent conductivity. For instance, Tabish *et al.* (2021) investigated graphene nanocomposites.[64]The non-invasive measurement of cholesterol using transdermal biosensors has demonstrated considerable potential.

4.1.3. Impedance spectroscopy. Impedance spectroscopy offers valuable insights into a system's electrical characteristics by measuring its impedance across a frequency range. It is particularly useful for detecting cholesterol levels in biological fluids. Impedance spectroscopy can be categorized into several types based on the sensing mechanism.

• *Enzyme-based impedance sensors*: these sensors catalyze reactions with cholesterol using enzymes like cholesterol oxidase, resulting in detectable electrical impulses. Lee *et al.* (2018) developed an enzyme-loaded paper with an impedimetric sensor that showed excellent sensitivity and specificity for detecting low levels of cholesterol in saliva.[57]

• *Non-enzymatic impedance sensors*: these sensors directly interact with cholesterol through the use of materials like conductive polymers or metal nanoparticles. Eksin *et al.* (2024) highlighted the value of non-invasive techniques using a cost-effective electrochemical impedance assay for determining salivary cholesterol levels.[65]

• *Advanced impedance techniques*: sophisticated methods such as multi-frequency impedance spectroscopy and 3D impedance mapping improve sensor detection. The potential of 3D electrical impedance spectroscopy for the early detection of circumstances related to cholesterol was highlighted by Abiri *et al.* (2022) during their exploration of the technique for *in situ* endoluminal mapping of metabolically active plaques.[66]Research has consistently demonstrated the effectiveness of impedance spectroscopy as a non-invasive method for cholesterol testing.

4.1.4. RF and microwave techniques. Radiofrequency (RF) and microwave procedures use electromagnetic waves to interact with biological tissues and evaluate different physiological characteristics. These techniques offer the advantage of real-time monitoring and non-invasiveness.[67] For instance, Spiliopoulos *et al.* (2017) demonstrated the potential of a microwave radiometry variation for non-invasive cholesterol detection as a diagnostic tool for vascular disorders.[68] Overall, RF and microwave techniques provide continuous, pain-free monitoring with good sensitivity and specificity. Microwave/RF methods are attractive for non-invasive sensing due to penetration depth and sensitivity to dielectric property changes. However, a key issue for cholesterol is specificity: most RF modalities measure bulk dielectric/thermal properties that can correlate with vascular inflammation, plaque composition, or temperature differences, rather than directly measuring serum cholesterol concentration. Earlier foundational work (*e.g.*, microwave radiometry in hypercholesterolemic models) showed that microwave radiometry could capture temperature differences that correlate with plaque-related parameters, supporting a role as a surrogate vascular assessment tool rather than a direct lipid assay.[69]

4.1.5. Ultrasound and MR imaging. Ultrasound imaging creates images of internal body structures by using high-frequency sound waves. Carotid Intima-Media Thickness (CIMT) measurement is indeed a widely used ultrasound technique to assess atherosclerosis, which is often associated with high cholesterol levels. This method measures the thickness of the arterial wall in the carotid arteries, which can provide valuable information about the presence and progression of plaque buildup. According to Kaspar *et al.* (2018), ultrasonography is a useful non-invasive imaging method for atherosclerosis, and it plays a crucial role in early diagnosis and monitoring.[70] Tissue stiffness is measured by ultrasound elastography, and this can be a sign of artery-clogging cholesterol. Elastography was utilized by Lee *et al.* (2020) to compare blood and lipidomic indicators in non-alcoholic fatty liver disease, highlighting its diagnostic utility.[71] MRI uses radio waves and magnetic fields to provide detailed images of body structures. It is renowned for having a high resolution and for not requiring ionizing radiation to photograph soft tissues. The MRI method, known as magnetic resonance spectroscopy, or MRS, is used to determine the chemical makeup of tissues. MRS was used by Lin *et al.* (2015) to measure liver fat and cholesterol, proving that it is a useful non-invasive detection method.[72] Tissue stiffness is measured by MRI elastography, which is more precise and detailed than ultrasonic elastography. Its potential for cholesterol detection was demonstrated by Makhija *et al.* (2020), who also emphasized its application in monitoring non-alcoholic fatty liver disease.[73]

4.1.6. Sweat, tear, saliva, and breath-based analysis. Over the past decade, biofluids like sweat, tears, and saliva have been used increasingly frequently for the non-invasive measurement of cholesterol.

• *Sweat analysis for cholesterol detection*: cholesterol is one of the many substances found in sweat that could potentially be used for non-invasive monitoring. Wearable sensors capable of continuous sweat composition monitoring are especially valuable. However, challenges such as sweat rate variability, potential interference from other sweat components, and the need for calibration can impact the accuracy of cholesterol measurements. In 2022, Tiwari *et al.* designed a wearable electrochemical biosensor that can identify low cholesterol concentrations in sweat. Because it provides real-time monitoring, this gadget is perfect for ongoing health assessments.[74] Tseng *et al.* (2021) examined sweat sample-based microfluidic paper-based medical diagnostic devices for human disorders. These gadgets are inexpensive and easy to operate, which increases their suitability for everyday observation.[75]

• *Tear analysis for cholesterol detection*: tears carry indicators that represent systemic health, such as cholesterol levels, and can be collected non-invasively, similar to sweat. While tears offer the advantage of continuous monitoring through smart contact lens technology, as demonstrated by Song *et al.* (2022), the relatively small volume of tears compared to sweat or saliva

Table 7 Comparison of non-optical cholesterol detection techniques based on accuracy, sample type, and validation approach

Method	Detection limit	Accuracy	Techniques	Advantages	Disadvantages	Cross-method evaluation	Sample type	Ref.
Biofluid-assisted electrochemical assays	Low	High	Voltammetry, amperometry	High specificity, low cost	Enzyme degradation, requires calibration	Compared with standard enzymatic colorimetric assays. Salivary cholesterol measured electrochemically correlated with serum cholesterol obtained *via* laboratory assays. Linear regression between electrochemical signal and enzymatic assay output	Saliva, sweat	59 and 87
Transdermal biosensors	Low	Moderate	Microneedles, iontophoresis	Continuous monitoring potential	Potential skin irritation	Interstitial fluid cholesterol correlated with blood cholesterol. Microneedle sensor readings compared against clinical lipid profile reports using electrochemical enzymatic assays	Interstitial fluid	63 and 64
Impedance spectroscopy	Low	High	Electrical impedance analysis	Real-time monitoring	Complex instrumentation	Electrical impedance changes correlated with biochemical cholesterol concentration. Validation against enzyme-based cholesterol assays	Blood, tissue	65 and 66
RF and microwave techniques	Moderate	Moderate	RF spectroscopy, microwaves	Continuous monitoring	Expensive	Microwave radiometry signals correlated with arterial lipid burden	Tissue	67 and 68
Ltrasound imaging	Moderate	High	CIMT, elastography	Detailed imaging	Requires trained operators	Structural imaging compared with blood lipid profiles. CIMT thickness correlated with LDL-C and total cholesterol	Tissue	88
MRI imaging	Low	Very high	MR spectroscopy	High resolution with detailed tissue analysis	Very expensive	MR-derived lipid content compared with biochemical assays. MRS-based cholesterol quantification validated against liver biopsy data and blood cholesterol levels	Tissue, blood	73 and 89
Sweat analysis	Low	High	Electrochemical sensors	Continuous monitoring	Sweat composition variability	Sweat cholesterol compared with blood cholesterol. Electrochemical sweat sensor outputs correlated with serum lipid panel values	Sweat	75
Tear analysis	Low	Very high	Smart contact lenses	Continuous monitoring	Tear fluid variability	Tear lipid composition compared with systemic lipid status. Tear cholesterol trends validated against blood cholesterol measurements	Tear	90
Saliva analysis	Low	High	Electrochemical sensors	Easy collection	Saliva composition variability	Salivary cholesterol compared with blood cholesterol. Enzyme-based saliva sensors validated against clinical biochemical assays	Saliva	80

Table 7 (*Contd.*)

Method	Detection limit	Accuracy	Techniques	Advantages	Disadvantages	Cross-method evaluation	Sample type	Ref.
Breath analysis	Low	Moderate	Electronic nose, VOC pattern analysis	Easy sample collection	Breath composition variability, environmental interference	Breath VOC signatures compared with blood cholesterol levels. Electronic-nose outputs validated against clinical biochemical lipid assays	Breath	83
NMR spectroscopy	Very low	Very high	High resolution NMR	Detailed metabolic profiling	Very expensive	Gold-standard comparison with mass spectrometry and enzymatic assays. Lipidomic NMR profiles cross-validated using clinical chemistry analyzers and GC/MS	Blood, urine	77 and 78

can pose challenges for biomarker detection.[76] Agrawal and Sivamani (2019) investigated tear-derived lipid mediators by non-invasive profiling. Their research emphasized the potential of tears to monitor cholesterol levels and other health indicators.[77]

- *Saliva analysis for cholesterol detection*: because of its constant production and ease of collection, saliva provides a practical, non-invasive approach for detecting cholesterol. Eom *et al.* (2020) developed a sensitive and non-invasive saliva-based cholesterol monitor by optimizing enzyme loading and platinum nanocluster composition.[78] This method improves the accuracy and consistency of cholesterol detection in saliva. Chu *et al.* (2022) demonstrated the feasibility of multimodal biosensor platforms for real-time monitoring of metabolic diseases *via* saliva. These devices can detect numerous biomarkers at once, including cholesterol, allowing for comprehensive health monitoring.[79]
- *Breath-based analysis for cholesterol detection*: emerging breath-based cholesterol detection approaches using electronic nose systems combined with machine-learning algorithms demonstrate promising discrimination capability but require further validation across diverse populations.[80]

4.1.7. Nuclear magnetic resonance. NMR spectroscopy uses the magnetic characteristics of atomic nuclei to obtain comprehensive information on molecular structures and compositions. This approach is especially useful for detecting and measuring cholesterol in various biofluids. Proton NMR, renowned for its sensitivity and ability to generate comprehensive metabolic profiles, has been widely employed for this purpose. Mika *et al.* (2017) illustrated the usage of ^{1}H NMR for normal serum lipidome analysis, emphasizing its usefulness in monitoring cholesterol level variations.[81] High-resolution NMR provides precise information about biofluids' biochemical makeup.

The classification of cholesterol detection techniques as invasive or non-invasive is determined strictly by the biological sampling process, rather than by the analytical instrumentation itself. From a clinical diagnostics and biomedical engineering perspective, invasive methods require penetration of the skin or blood vessels to obtain samples. Consequently, conventional NMR-based lipid profiling performed on venous blood or serum

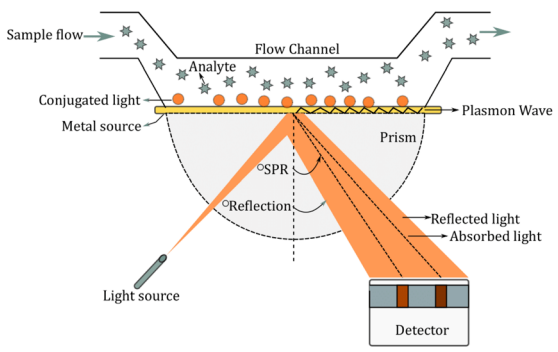

Fig. 4 Schematic representation of surface plasmon resonance showing light matter interaction at the sensor surface.

Table 8 Summary of research studies employing surface plasmon resonance for cholesterol sensing and analysis

Authors	Year	Key findings	Cross-method evaluation	Wavelength	Advantages	Disadvantages	Ref.
Yanase *et al.*	2014	FTIR-SPR for monitoring cell occupancy and membrane composition including cholesterol	SPR resonance shifts correlated with biochemical and spectroscopic analyses	<500 nm	Detailed biochemical membrane analysis	Limited to cell-based studies	80
Immanuel *et al.*	2018	Colorimetric cholesterol detection using TPU nanofibre/cellulose acetate membrane	Validated against standard enzymatic cholesterol assays	550 nm	Simple and cost-effective	Limited detection range	81
Parkkila *et al.*	2018	Dual-wavelength SPR and microbalance lipid bilayer characterization	Cross-validated with quartz crystal microbalance data	670, 830 nm	Detailed biophysical insight	Not cholesterol-specific	82
FS Jahed *et al.*	2020	Review of SPR applications in healthcare including cholesterol	Compared with electrochemical and spectroscopic methods	Not mentioned	Highlights SPR versatility	No experimental validation	83
Jebelli *et al.*	2020	SPR biosensors for microRNA detection applicable to cholesterol	Benchmarked against fluorescence and electrochemical biosensors	650 nm	High sensitivity and multiplexing	Focused on microRNAs	84
Kumar *et al.*	2021	Review of optical techniques including SPR/LSPR for cholesterol	Compared with Raman, IR and electrochemical methods	Not mentioned	Comprehensive overview	Broad focus	17
Cheng *et al.*	2022	Cholesterol recognition assay for exosome detection	Compared with nanoparticle biochemical assays	Not mentioned	High sensitivity	Exosome-focused	85
Choudhary *et al.*	2023	Point-of-care SPR sensor for myocardial infarction	Correlated with standard clinical biomarkers	670 nm	High sensitivity	Not cholesterol-specific	86
Negahdari *et al.*	2023	MIM plasmonic biosensor for multi-biomarker detection	Simulation benchmarked with reported SPR data	5333 nm	Multi-analyte design	Simulation only	87
Bernardo *et al.*	2024	Peptide-based plasmonic biosensor for free cholesterol	Validated using competitive biochemical assays	569 nm	Label-free detection	Preprint stage	88
Zheng *et al.*	2024	Dual-parameter in-fiber SPR cholesterol sensor	Calibrated using standard cholesterol solutions	500 nm	Temperature-compensated sensing	Complex demodulation	89
Rafiee *et al.*	2024	PIT-based plasmonic hybrid biosensor	Numerical comparison with reported SPR sensors	6045 nm	Very high predicted sensitivity	No experimental data	90
Zhang *et al.*	2025	Fiber SPR cholesterol biosensor with Ag and PDA layers	Validated using standard cholesterol solutions	3261 nm	Low-cost fabrication	Biofouling risk	91
Fang *et al.*	2025	TFBG-based SPR cholesterol biosensor	Benchmarked with planar SPR sensors	1480–1600 nm	Ultra-sensitive detection	Requires precise coating	92

samples remains invasive, despite the non-destructive nature of the NMR measurement, because blood extraction is required.[82] NMR-based cholesterol assessment is considered non-invasive in this review only when applied to biofluids that can be collected without breaching biological barriers, such as saliva or urine. Saliva collection is entirely needle-free, painless, and poses minimal risk of infection, making it a well-established non-invasive sampling approach in clinical metabolomics. Several studies have demonstrated the feasibility of ^{1}H NMR analysis of saliva for comprehensive metabolic profiling, including lipid- and cholesterol-associated resonances, with minimal sample preparation. Recent reviews and methodological studies have further confirmed the robustness and reproducibility of saliva-based NMR metabolomics for non-invasive clinical assessment. From a technical standpoint, advances in high-field ^{1}H NMR spectroscopy, optimized pulse sequences, efficient water suppression strategies, and multivariate spectral analysis have enabled reliable detection of low-concentration lipid-related metabolites in complex biofluids such as saliva.[83]

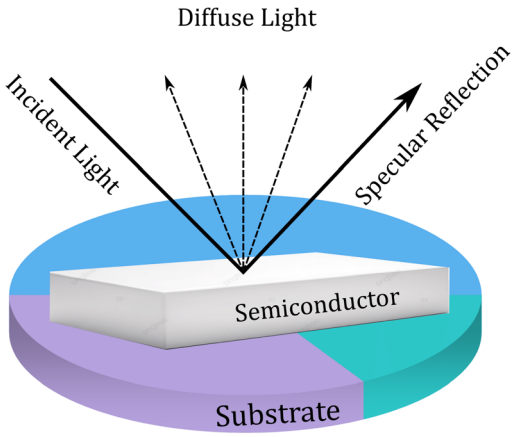

Fig. 5 Working principle of diffuse reflectance spectroscopy measuremnet principle.

Table 9 Summary of reported diffuse reflectance spectroscopy techniques investigated for cholesterol measurement

Authors	Year	Key findings	Wavelength	Advantages	Disadvantages	Ref.
Hou *et al.*	2016	Non-invasive rapid detection of skin cholesterol using diffuse reflectance spectroscopy	500–600 nm	Rapid, non-invasive	Specific wavelength not clearly stated	95
Hou *et al.*	2017	Demonstrated non-invasive detection of skin cholesterol using diffuse reflectance spectroscopy	475–780 nm	Non-invasive, convenient	Requires hand-held instrument	96
Chao *et al.*	2018	Proposed a non-invasive skin cholesterol detection method based on diffuse reflectance spectroscopy	380–780 nm	Non-invasive, structured method	Requires specific procedure and equipment	90
Ni *et al.*	2021	Developed a non-invasive method for skin cholesterol detection using diffuse reflectance spectroscopy	400–780 nm	Non-invasive, *in situ* detection possible	Requires detection reagent, limited to skin epidermis	97
Rickard, A. G. *et al.*	2023	Clinical evaluation of diffuse reflectance spectroscopy for quantifying tissue optical properties *in vivo*, establishing feasibility in human diagnostics	400–1000 nm	Clinically validated DRS; non-invasive; real-time capability	Focus on optical properties, not biochemical cholesterol quantification	98
Buendía-Avilés, S. *et al.*	2024	Validated diffuse reflectance spectroscopy using vegetal phantoms mimicking skin optical behavior, supporting calibration strategies	400–1000 nm	Low-cost DRS validation; improves accuracy of reflectance-based systems	Phantom-based only; no cholesterol or lipid measurement	99
Liang G. *et al.*	2025	Proposed UV–Vis–NIR spectroscopy combined with deep neural networks to quantify serum biochemical indexes including cholesterol	200–2500 nm	Rapid, reagent minimal optical approach	Not pure DRS; uses broad UV–Vis–NIR spectra	100
Savelieva, T. *et al.*	2025	Reviewed machine-learning assisted diffuse reflectance spectroscopy methods for clinical diagnostics, highlighting future trends	400–900 nm	Comprehensive methodological overview; highlights future DRS directions	Review paper; no experimental cholesterol data	101
Serrano, D. *et al.*	2025	Compared mid-IR and near-IR spectroscopy for serum biomarker prediction, demonstrating feasibility of reflectance-based biochemical analysis	780–25000 nm	Reagent-free; clinically relevant serum analysis	Cholesterol not directly targeted; not pure DRS	102

In typical salivary NMR workflows, sample preparation is limited to simple steps such as centrifugation and buffering prior to spectral acquisition, thereby avoiding invasive pre-processing while maintaining analytical rigor and quantitative reliability.[84] Recent state-of-the-art analyses have emphasized the growing clinical relevance of saliva NMR metabolomics, particularly for cardiometabolic and lipid-related disorders.[85] Speyer and Baleja (2021) proposed the use of high-resolution NMR to diagnose metabolic diseases, which can also be used to monitor cholesterol levels.[86] Table 7 presents a comparison of non-optical cholesterol measurement techniques.

4.2. Optical techniques

This section provides a brief explanation of seven optical techniques, offering an overview of their characteristics, benefits, and drawbacks.

4.2.1. Surface plasmon resonance. Surface Plasmon Resonance (SPR) is an optical technique that measures changes in the mass of biomolecules bound to a metal film. It operates based on the principle of total internal reflection, where light traveling through a dense medium (like glass) is reflected back when it encounters a less dense medium (like liquid).[91] When biomolecules bind to the metal film, they alter its refractive index, which in turn affects the properties of the reflected light. This change in the reflected light can be measured and used to quantify the binding of biomolecules to the surface.[92]

Polarized light is directed at a sensor, where an immobilized molecule binds with a free analyte in solution. Analyte binding causes a change in refractive index near the sensor surface, which allows for real-time monitoring of molecular interactions as shown in Fig. 4.

SPR is a promising technology for detecting non-invasive biomarkers like cholesterol. SPR provides non-invasive, label-free detection and real-time monitoring, but it requires specialized equipment, is confined to surface interactions, and requires metal-coated surfaces. Table 8 shows a summary of the SPR technique used for cholesterol detection.

Researchers explored the use of Surface Plasmon Resonance (SPR) for cholesterol detection by immobilizing cholesterol binding molecules, such as antibodies or enzymes, on a sensor chip. When cholesterol interacts with these immobilized molecules, it causes a measurable shift in the resonance frequency, indicating the presence and potentially the concentration of cholesterol. Although SPR is a label-free and highly sensitive technique, it is unsuitable for non-invasive cholesterol testing as it requires direct contact between the sample and the sensor. This necessitates blood extraction, cholesterol separation, and exposure to the sensor chip.

4.2.2. Diffuse reflection spectroscopy. Diffuse Reflection Spectroscopy (DRS) determines the temperature of a semiconductor substrate by analyzing its optical absorption edge, which shifts with temperature. A white light source illuminates the sample from the outside, and the light is partially reflected on the front surface and partially transmitted through the substrate.[93] Inside, the light is diffusely distributed. Unlike specular reflection, which occurs at a specific angle equal to the incident angle, diffuse reflection spreads out. A detector located away from the specular reflection captures this scattered light, providing information about the substrate's band gap which is related to the material's electronic properties and changes with temperature.[94] To reduce background noise and improve measurement accuracy, a lock-in amplifier is employed. The Fig. 5 illustrates the concept of diffuse reflectance spectroscopy.

Table 9 shows a summary of the DRS technique used for cholesterol detection.

Overall, DRS is a promising field of study for non-invasive cholesterol testing. Although not yet clinically validated, the technology offers the potential for a convenient and painless method of monitoring cholesterol levels in the future. However, challenges remain, including distinguishing the cholesterol signal from other skin components and ensuring an accurate translation of reflectance patterns into cholesterol concentrations.

4.2.3. Fluorescence spectroscopy. Fluorescence spectroscopy (which is also called as fluorimetry or spectrofluorometry) is a type of electromagnetic spectroscopy that measures fluorescence in a sample.[103] It entails utilizing a beam of light, usually ultraviolet light, to excite the electrons in molecules of specific chemicals and cause them to emit light. Fluorescence spectroscopy is an investigative method that uses the fluorescence properties of the subject under examination to make quantitative measurements of chemical products. Fluorescence spectroscopy studies molecules that glow under UV light, revealing the structure and composition of organic matter in various materials.[104] This technique has become increasingly important with the advent of lasers in the 20th century. The basic setup of fluorescence spectroscopy consists of a light source, two monochromators, a sample holder, and a detector. A monochromator is a device that selects a specific wavelength of light. The first monochromator selects the excitation wavelength, while the second analyzes the emitted light. The detector is positioned at a 90-degree angle to the excitation beam. A xenon arc lamp, which emits UV, visible, and near-

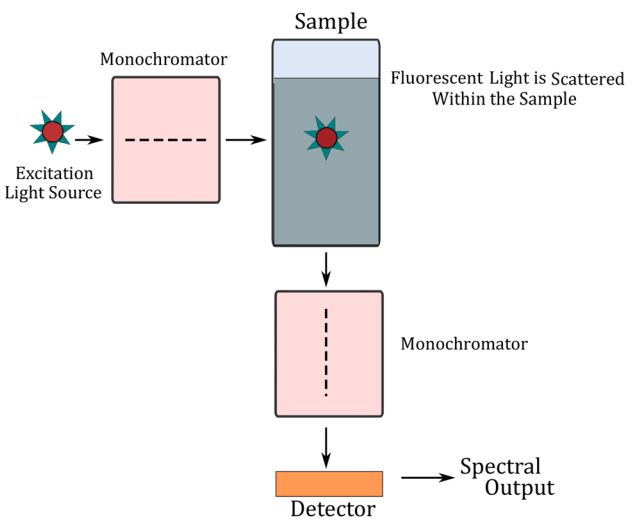

Fig. 6 Optical layout of fluorescence spectroscopy showing excitation, emission, and detection paths.

infrared radiation, sends light to the excitation monochromator. The light is then sent into the sample chamber *via* a customized fluorescence cuvette. Excited sample molecules emit light, which is detected at right angles by the emission monochromator. A photomultiplier tube measures light intensity, whereas the output current reflects fluorescence extent.[105] The process of fluorescence spectroscopy is shown in Fig. 6.

Recent studies in fluorescence spectroscopy for cholesterol detection show that probe specificity is crucial for reliable measurements, especially in complex biological samples such as serum, saliva and skin. Recent studies in fluorescence spectroscopy for cholesterol detection show that probe specificity is crucial for reliable measurements, especially in complex biological samples such as serum, saliva and skin. Fluorescence-based cholesterol sensors are generally grouped into enzyme-assisted systems, nanomaterial-based probes, and molecular-recognition probes, with each type using a different mechanism to selectively respond to cholesterol. Among nanomaterial-based probes, heteroatom-doped carbon dots are widely used because their surface chemistry can be easily tuned and they provide strong fluorescence signals. In particular, nitrogen–cobalt co-doped carbon dots show high selectivity toward cholesterol due to coordination between cobalt sites and the hydroxyl group of cholesterol, leading to sensitive and repeatable fluorescence changes.[106] Ratiometric fluorescence systems combining carbon dots with gold nanoclusters improve detection accuracy by reducing background interference and enabling internal calibration in serum and skin samples.[107,108] In addition, near-infrared fluorescent nanomaterials and defect-engineered carbon structures improve tissue penetration and cholesterol selectivity, making them suitable for non-invasive sensing.[109] Molecular-recognition-based fluorescent probes further enhance selectivity by directly interacting with cholesterol molecules. Small-molecule fluorescent probes allow enzyme-free cholesterol detection with low cross-reactivity, while β-cyclodextrin-functionalized gold nanoclusters selectively bind cholesterol through host–guest interactions. Dual-mode and ratiometric sensors, including upconversion nanoparticle-based probes and zinc/carbon-dot hybrid systems, improve measurement stability by compensating for effects such as photobleaching and sample variability.[110,111]

Table 10 shows a summary of the fluorescence spectroscopy technique used for cholesterol detection.

Fluorescence spectroscopy holds great promise for non-invasive cholesterol detection. While challenges remain in developing highly precise probes and accurately converting

Table 10 Summary of research studies on fluorescence-based approaches for cholesterol detection

Authors	Year	Key findings	Wavelength	Advantages	Disadvantages	Ref.
Huang *et al.*	2019	Nitrogen and cobalt co-doped fluorescent magnetic carbon dots used for sensitive cholesterol detection	465–540 nm	High sensitivity and selectivity	Lack of specific wavelength information	112
Wu *et al.*	2021	Rapid, non-invasive detection of skin cholesterol using fluorescent spectrometry	462–520 nm	Rapid, non-invasive, uses fluorescent spectrometry	Lack of detailed wavelength information	113
Hu *et al.*	2021	High sensitivity and selectivity in detecting cholesterol using ratiometric fluorescence probe	400 nm	High sensitivity and selectivity; suitable for human serum samples	Lack of specific wavelength information	114
Lai *et al.*	2021	Non-invasive skin cholesterol testing as a proxy for LDL-C levels	Not mentioned	Non-invasive; potential proxy for LDL-C levels	Potential interference from other skin components	115
X. Liu *et al.*	2024	Portable fluorescent color detection system for rapid enzyme-free detection of serum cholesterol using β cyclodextrin capped gold nanoclusters	365–700 nm	Rapid and highly sensitive; enzyme-free; good agreement with standard methods	Wavelength specifics not fully defined; color-sensor based	116
V. Rubio *et al.*	2024	Designed fluorescent cholesterol analogs for live-cell imaging, enabling visualization of cholesterol trafficking in cells	488–650 nm	Environment-sensitive; enables live-cell cholesterol imaging	Not quantitative for serum; requires fluorescence microscopy	117
S. Basu	2024	Ratiometric near-infrared fluorescence probe based on defect-engineered SWCNTs for cholesterol detection	Near-IR (∼1000–1400 nm)	Ratiometric signal reduces noise; high sensitivity; deeper probing	Complex nanomaterial design; specialized instrumentation	118
P. Tingyuan *et al.*	2024	Dual-readout colorimetric and fluorescent sensor using upconversion nanoparticles and TMB quenching strategy	450–980 nm	Dual readout improves robustness; fluorescence enhances sensitivity	Requires optimized reaction conditions; fluorophore-specific	119
J. Li *et al.*	2025	Cholesterol esterase- responsive near-infrared fluorescent probe (NR-CHE) with selective turn-on fluorescence	650–780 nm	High selectivity; NIR reduces background interference	Requires enzymatic activation; specific probe synthesis	120

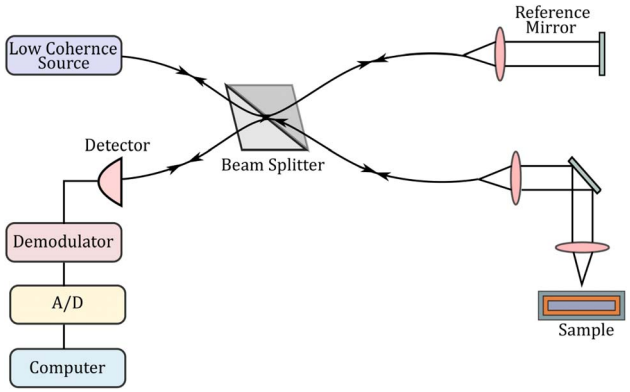

Fig. 7 Schematic representation of the optical coherence tomography system sowing reference mirror and sample arms.

fluorescence data into cholesterol levels, continued research in probe design, data processing, and calibration procedures could transform this technology into a valuable tool for future cholesterol monitoring.

4.2.4. Optical coherence tomography. Optical Coherence Tomography (OCT) is a non-invasive imaging technique. OCT uses low-coherence interferometry [121] to create detailed images of tissues by sending light into the tissue and comparing it to light reflected from a reference mirror, as shown in Fig. 7. The light used in OCT is unique due to its short coherence length, meaning it can only interfere with light that has traveled nearly the same distance. This property allows OCT to measure interference patterns and accurately determine the depth of various tissue structures, resulting in high-resolution images.[122]

OCT is a non-invasive imaging technology for obtaining high-resolution cross-sectional pictures of biological tissues. It operates similarly to ultrasonic imaging but utilizes light instead of sound. OCT is commonly utilized in ophthalmology, cardiology, and dermatology.[123] OCT was first demonstrated *in vitro* by an MIT and Harvard Medical School team in 1991. Since its inception, OCT has evolved into a valuable diagnostic tool across various medical specialties. OCT makes use of a broadband light source or a swept-source laser, which splits the light into reference and sample arms. Light backscattered from the sample and reflected from the reference arm is combined to form an interference pattern, which produces depth-resolved pictures. OCT identifies and quantifies cholesterol deposits by detecting unique optical features of plaques that separate healthy tissue, lipid-rich plaques, and fibrous caps. Table 11 represents a summary of the OCT technique used for cholesterol detection.

Researchers have investigated employing OCT to locate cholesterol crystals within plaque accumulation in arteries during invasive operations, but its limited tissue penetration

Table 11 Summary of optical coherence tomography methods applied to cholesterol-related plaque characterization

Authors	Year	Key findings	Wavelength	Advantages	Disadvantages	Ref.
Matthäus *et al.*	2018	Characterization of early plaque formations using Raman probe spectroscopy combined with OCT	785 nm	Early detection; multimodal approach	Limited to animal models	124
Araki *et al.*	2022	OCT-based assessment and intervention in coronary atherosclerosis	1300 nm	Comprehensive review of OCT in coronary atherosclerosis	Focuses on coronary interventions rather than cholesterol	125
Afsharan *et al.*	2023	Retinal blood vessel wall measurements using polarization-sensitive OCT for diabetes assessment	840 nm	Non-invasive; detailed structural assessment	Specific to diabetes; not cholesterol-focused	126
Chen *et al.*	2024	Detection of atherosclerotic plaques using HDL-like porphyrin nanoparticles with dual-modality OCT and fluorescence imaging	1310 nm	Improved detectionvia dual-modality imaging	Requires specialized nanoparticles	127
Nelles, G. *et al.*	2024	Cholesterol crystals (CCs) in culprit lesions linked to high-risk plaque features and worse cardiovascular outcomes	Intravascular OCT (~1300 nm)	High-resolution plaque imaging; CC identification	Invasive; no direct biochemical quantification	128
Lee, J. *et al.*	2024	Plaque feature analysis *via* intravascular OCT to predict long-term cardiovascular mortality	IVOCT (~1300 nm)	Predictive plaque metrics; standardized analysis tools	Not exclusively cholesterol-specific	129
Di Muro, F. M. *et al.*	2024	OCT plaque morphology correlated with Lp(a) levels in ACS patients	Intravascular OCT (~1300 nm)	Links lipid biomarkers with OCT features	Indirect cholesterol association	130
Deng, C. *et al.*	2025	Predictive model linking cholesterol crystals with plaque vulnerability features using OCT	Intravascular OCT (~1300 nm)	Quantitative risk modeling	Model-based; invasive; not biochemical	131
Yang, D. *et al.*	2025	Visualization of cholesterol crystals and plaque rupture with lipid ratio associations using OCT imaging	Near-IR OCT (~1300 nm)	Combines lipid ratios with morphological features	Early-stage study; limited validation	132

depth makes it unsuitable for non-invasive examinations. OCT is primarily used on light-accessible tissues, such as the eye, and is also employed during minimally invasive operations.

4.2.5. Photoacoustic spectroscopy. PAS is a non-invasive method that combines optical and acoustic approaches. Photoacoustic (PA) techniques provide molecular sensitivity by converting absorbed optical energy into acoustic waves, enabling deeper probing than purely optical reflectance and offering spectral selectivity for chromophores (*e.g.*, lipids) when combined with multispectral excitation. Recent literature emphasizes PA as a safe non-ionizing modality capable of molecular quantification and spectral contrast when the excitation wavelength is tuned across absorption bands. In particular, PA and absorption spectroscopy imaging approaches have been reviewed as non-invasive molecular quantification strategies, with ongoing improvements in illumination sources, detection architectures, and spectral interpretation methods. On portability and device development, broader PA technology trends show rapid progress toward compact illumination (*e.g.*, LED-based and diode-based sources) and integration strategies that reduce system cost/size while improving practicality for clinical workflows. A recent review discusses advances in photoacoustic imaging hardware including prospects for compact excitation sources and practical deployment considerations.[133] In addition, clinical-leaning PA device form factors such as flexible or patch-like architectures (*e.g.*, integrated arrays with compact emitters) illustrate the larger momentum toward wearable/portable PA platforms, which is relevant when discussing translation of PA methods toward point-of-care settings, even if many current implementations focus on vascular imaging rather than direct blood lipid quantification.[134] When a sample absorbs laser light, it produces localized heat,[135] causing thermo elastic expansion and the production of ultrasonic waves (acoustic signals). By analyzing the characteristics of these acoustic signals, such as frequency and amplitude, researchers can identify the specific components present in the sample.[136] The basic block diagram of photoacoustic spectroscopy is shown in Fig. 8.

PAS relies on the PA process, which generates sound waves by light absorption in a sample. A light source, often a laser, is focused onto the sample. At specified wavelengths, molecules in the sample absorb light, converting it into heat. By modulating the light while maintaining a constant sample volume, periodic heating occurs, creating pressure waves detectable by a microphone. A tunable laser source is often selected for cholesterol detection in PAS because it can target specific wavelengths absorbed by cholesterol molecules. A modulator may be used to modulate the laser beam at a specified frequency. The modified laser beam is then directed toward the skin's surface using a light delivery system, which may include lenses or fibers. The PAS measurement focuses on the skin area containing the cholesterol. A sensitive microphone near the skin detects pressure waves from cholesterol molecules absorbing light and expanding thermally. A pre-amplifier boosts this weak signal, and if a modulator is used, a lock-in amplifier filters noise and amplifies the signal at the modulation frequency. The amplified signal is converted into digital data for analysis. Specialized

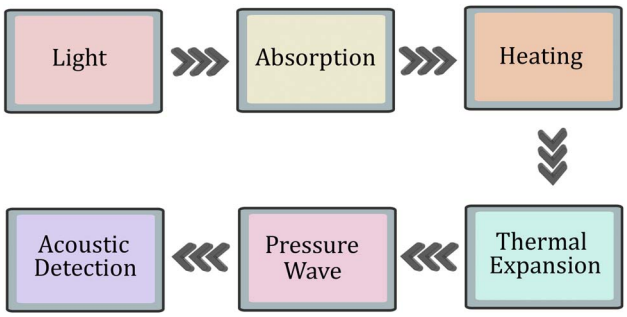

Fig. 8 Block diagram outlining the signal flow from light source to acoustic detection in a photoacoustic cholesterol sensing system.

software processes this data to determine cholesterol concentration by examining factors like signal amplitude, frequency response, and phase shift at wavelengths with high cholesterol absorption. However, system cost, laser safety considerations, and complex signal interpretation remain major barriers to widespread adoption. Consequently, current research emphasizes hybrid optical-acoustic architectures as experimental platforms rather than clinically deployable solutions, underscoring an ongoing debate between sensitivity enhancement and practical scalability. Recent developments in photoacoustic techniques have increasingly focused on cholesterol-related lipid and plaque characterization, leveraging the strong lipid absorption bands in the near-IR/SWIR region and combining optical contrast with ultrasound scale imaging depth. Notably, dual-modal photoacoustic and ultrasound implementations have been highlighted for lipid-rich plaque assessment, where photoacoustic contrast provides chemical sensitivity while ultrasound improves structural localization and workflow feasibility. Current research trends emphasize compact light delivery, improved wavelength selection for lipid sensitivity, and integration strategies that support portability and clinical workflow alignment, positioning PAS as a promising emerging modality for cholesterol-linked vascular risk assessment. In some PA works, it is reported as "cholesterol detection" may target cholesterol-related plaque components rather than serum cholesterol concentration. As an example, Salih *et al.* (2023) developed a PA based approach aimed at detecting cholesterol-related vessel characteristics and employed novel signal processing to improve sensitivity, reinforcing the importance of algorithmic processing in PA pipelines.[137] PA is currently more mature for lipid-rich plaque characterization and tissue composition mapping, whereas non-invasive serum cholesterol quantification remains challenging due to calibration constraints and physiological confounders (skin, perfusion, scattering, inter-subject variability). Table 12 shows a summary of the PAS technique used for cholesterol detection.

4.2.6. Raman spectroscopy. Raman spectroscopy has emerged as a powerful technique for studying various biological components such as albumens, enzymes, chromosomes, nucleoproteins, sterols, biological membranes, and arbohydrates.[148] The technique involves the interaction of incident photons with molecules. The energy from these photons excites

Table 12 Summary of photoacoustic based methods reported for cholesterol and lipid detection

Authors	Year	Key findings	Wavelength	Advantages	Disadvantages	Ref.
Jansen *et al.*	2014	Spectroscopic intravascular photoacoustic imaging of lipids in atherosclerosis	1200 nm, 1700 nm	Detailed lipid mapping	Limited depth penetration	138
Dasa *et al.*	2019	Multispectral photoacoustic sensing for accurate glucose and cholesterol monitoring using supercontinuum laser	1540–1840 nm	Accurate analyte identification	High cost of supercontinuum laser	139
Iskander-Rizk *et al.*	2021	Spectral differentiation of cholesteryl compounds	1720 nm	Depth resolution; compound differentiation	Requires spectral separation	140
Jin *et al.*	2022	Non-invasive monitoring using photoacoustic spectroscopy	1714 nm, 1734 nm	Potential for continuous monitoring	High initial system cost	141
Tsai, W. Y. *et al.*	2023	Photoacoustic features correlated with multiple biochemical blood parameters including cholesterol	680–1064 nm	Quantitative correlation with lipid biomarkers	Not pure PA spectral cholesterol quantification	142
Schneider, M. K. *et al.*	2023	NIR auto-photoacoustic imaging combined with ultrasound to differentiate plaque vulnerability	700–900 nm	Improved plaque characterization	Indirect cholesterol specificity	143
Zhou, W. *et al.*	2023	Review of multimodal photoacoustic imaging methods for identifying vulnerable plaques	1210–1720 nm	Comprehensive methodological overview	Review paper; no primary data	144
Riksen, J. J. M. *et al.*	2024	Multispectral PA analysis differentiated lipid-rich regions in human carotid plaques	850–1250 nm	Discriminates lipid spectral signatures	Ex vivo study; not clinically validated	145
Yu, C. *et al.*	2024	Combined near-IR photoacoustic imaging with ultrasound to detect lipid-rich plaques	700–900 nm	Non-invasive plaque characterization	Does not isolate cholesterol specifically	146
Wang, K. *et al.*	2024	PA probe (Hcy-CE) enabled *in vivo* imaging of cholesteryl esters in animal models	700–750 nm	Targeted CE visualization	Requires chemical probe; not label-free	147

the molecule's vibrational modes. A laser beam is directed toward a sample, and scattered light is collected and evaluated[149] as shown in Fig. 9. The Raman spectrum, a plot of the intensity of scattered light as a function of wavelength, provides information about the sample's chemical composition and structure. This approach is frequently applied in various domains, including chemistry, biology, and materials research.

In Raman scattering,[150] the energy of the scattered photon differs from that of the incident photon. When a molecule in its ground state absorbs energy from an incident photon, it transitions to a higher vibrational energy level. The scattered photon in this case, known as the Stokes–Raman scatter, has less energy than the incident photon. The Raman photon has a higher energy (*i.e.*, the photon's emitted wavelength moves to lower values). The term "Anti-Stokes" refers to this kind of Raman radiation. It is clearly shown in Fig. 10.

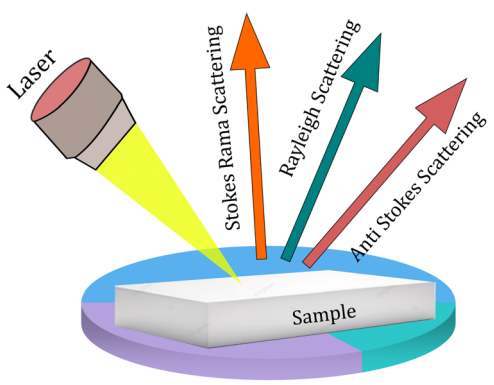

Fig. 9 Conceptual illustration of Raman spectroscopy setup demonstrating the interaction between incident light and molecular vibrations.

Table 13 shows a summary of the Raman Spectroscopy technique used for cholesterol detection.

Skin contains numerous chemicals that can interfere with the Raman signal generated by cholesterol. Isolating the weak cholesterol signal from the complex biological background noise within tissue presents a significant challenge. Developing specialized procedures and advanced data analysis methodologies is essential to ensure accurate cholesterol measurement. In practice, achieving reliable *in vivo* Raman cholesterol quantification faces several well-documented challenges that must be explicitly acknowledged. First, melanin and other endogenous chromophores in skin produce intense broadband autofluorescence that can overwhelm the comparatively weak Stokes Raman signal from cholesterol, particularly at excitation wavelengths below 785 nm.[158] Second, the characteristic Raman bands of cholesterol, particularly the C–H stretching region (2800–3000 cm^{-1}) and the C=C stretching peak (1670 cm^{-1}), overlap with bands from other skin lipids, including ceramides, triglycerides, and phospholipids, requiring advanced spectral

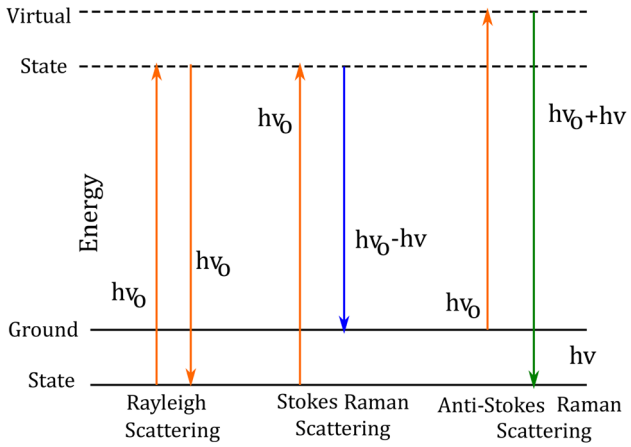

Fig. 10 Energy level diagram showing molecular transitions during the scattering process.

deconvolution or multivariate chemometric models to isolate the cholesterol-specific contribution.[87,159] Third, the *in vivo* signal-to-noise ratio (SNR) for skin-based Raman cholesterol detection is inherently low because the accessible epidermal cholesterol concentration is significantly lower than blood cholesterol levels, and light scattering in turbid tissue further attenuates the Raman return signal.[160]

4.2.7. Infrared spectroscopy. Infrared spectroscopy[161] is a technique for identifying and studying substances by analyzing their interaction with infrared radiation. This approach is primarily concerned with the infrared part of the electromagnetic spectrum, which is divided into three regions: Near-Infrared (NIR),[162] which ranges from 750 to 2500 nm; Mid-Infrared (MIR),[163] which spans from 2500 to 25 000 nm; and Far-Infrared (FIR)120, which ranges from 25 000 to 1 000 000 nm. One of its great advantages is that virtually any sample may be studied in any state. Liquids, solutions, pastes, powders, films, fibers, gases, and surfaces can all be examined with a judicious choice of sampling technique. Unlike some other methods, IR spectroscopy doesn't require special sample preparation, allowing direct analysis. It provides a comprehensive spectrum within seconds, revealing information about the entire frequency range.[164] When infrared radiation passes through a sample, certain wavelengths are absorbed, corresponding to the vibrational frequencies of the molecule's bonds. These vibrations, including stretching and bending, are unique to each bond and functional group. An infrared spectrometer measures light intensity, producing a spectrum with peaks that identify molecular structures as shown in Fig. 11.

Non-invasive IR spectroscopy for cholesterol detection holds promise for routine cholesterol monitoring in clinical settings and home use. It can potentially lead to more efficient management of cardiovascular diseases by enabling regular monitoring of cholesterol levels without the need for blood draws. Infrared spectroscopy distinguishes itself from other optical methods through its molecular specificity, non-destructive nature, and wide range of applications. Its

Table 13 Summary of reported Raman spectroscopy-based approaches for cholesterol sensing in biological samples

Authors	Year	Key findings	Wavelength	Advantages	Disadvantages	Ref.
Schie *et al.*	2013	Assessed cholesterol and its esters in human cells using Raman scattering techniques	785 nm	Label-free, high sensitivity	Limited to *in vitro*	89
Wang *et al.*	2013	Demonstrated quantitative imaging of cholesterol in tissues using stimulated Raman scattering microscopy	700–1300 nm	Fast, quantitative, label-free	Equipment cost, complexity	151
Borges *et al.*	2015	Detected spectral differences in lipid and glucose components in serum	830 nm	Quantifies cholesterol	Requires serum samples	152
Lee *et al.*	2015	Developed a method for imaging cholesterol in live cells using Raman spectroscopy	707 nm	Non-invasive imaging	Requires specialized equipment	88
Meksiarun *et al.*	2016	Quantitatively analyzed molecular composition of fat using Raman spectroscopy	785 nm	Non-invasive, quantitative analysis	Limited to subcutaneous adipose tissues	153
Parachalil *et al.*	2020	Discussed the benefits of using Raman for analyzing plasma/serum	785 nm	Detailed chemical analysis	Requires liquid samples	154
Beton mysuru *et al.*	2023	Reported a Raman spectroscopic modality for tracking cholesterol	5555 nm	Enables chemical imaging of cholesterol changes in cells	Limited to exvivo tissue	155
Jia *et al.*	2024	Raman spectral information identified plaque depositions consisting of lipids, triglycerides, and cholesterol	785 nm	Deep organ tissue characterization	High cost of equipment	156
Davydova *et al.*	2025	Applied SERS of blood serum to differentiate clinical atherosclerosis including cholesterol	785 nm	Enables disease satisfaction linked to lipid metabolic	Indirect cholesterol inferences	157

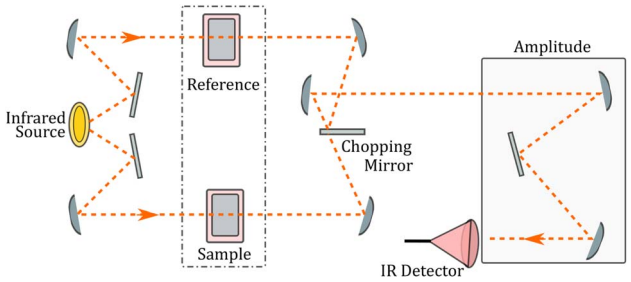

Fig. 11 Basic infrared spectroscopy diagram showing radiation generation, sample absorption, and signal detection.

superior ability to provide detailed molecular fingerprints and perform quantitative analysis makes it indispensable in biomedical research and clinical diagnostics. While other optical methods have their unique advantages, IR spectroscopy's versatility and precision offer unparalleled benefits, particularly in non-invasive applications. As technology advances, the integration of IR spectroscopy with other analytical techniques will further enhance its utility, solidifying its role as a cornerstone in biomedical science.

5. Comparative analysis of techniques

Non-optical approaches, such as electrochemical sensors and chromatographic procedures, provide practical benefits such as ease of use and cost savings. Electrochemical sensors, for example, can be developed for point-of-care applications, delivering immediate findings with little sample preparation. These approaches are frequently more robust under varied environmental circumstances and can be built into portable equipment for on-site testing. However, non-optical approaches may suffer from specificity and sensitivity concerns, especially in complex biological matrices where interference from other substances can result in false results. On the other hand, Optical techniques, such as Raman and infrared spectroscopy, are well-known for their sensitivity and specificity. These techniques enable real-time monitoring and can provide extensive information about the molecular makeup of cholesterol. Their non-invasive nature makes them ideal for continuous monitoring applications, such as wearable devices. However, these procedures frequently confront difficulties due to expense, complexity, and the requirement for specialized equipment and trained personnel. Furthermore, biological variability and the influence of external variables (for example, temperature and humidity) might have an impact on optical measurement accuracy. The comparative study takes into account the environment in which each method is implemented. For example, in therapeutic situations where precision is critical, optical procedures may be favoured, despite their increased cost. In contrast, in community health settings where rapid screening is required, non-optical technologies may be more appropriate due to their accessibility and ease of use. Finally, the decision between optical and non-optical technologies is determined by the individual monitoring scenario requirements, such as required accuracy, cost limits, and the necessity for real-time data. This section emphasizes the necessity of selecting the proper approach based on the context of use, ensuring that the chosen method is consistent with the objectives of efficient cholesterol monitoring and management.

Table 14 shows a consolidated quantitative comparison of both optical and non-optical methods for cholesterol detection.

6. Wearable sensors and global applicability of point-of-care diagnostics

Recent advances in wearable sensor technologies have significantly expanded their role in cardiovascular disease (CVD) monitoring and risk assessment, particularly in the context of cholesterol-associated disorders. Modern wearable platforms integrate electrocardiography (ECG), photoplethysmography (PPG), heart-rate variability (HRV), arterial stiffness estimation, and motion-corrected physiological sensing to enable continuous, non-invasive cardiovascular monitoring in real-world settings.[166–168] Systematic and scoping reviews published during this period demonstrate that wearable cardiac devices have progressed beyond consumer wellness tools and now exhibit clinically relevant accuracy for long-term rhythm monitoring, early detection of cardiac abnormalities, and longitudinal cardiovascular risk stratification.[169–171]

Advances in flexible electronics, electrochemical sensing, and multimodal mechanical sensors have further enhanced signal stability, miniaturization, and user comfort, enabling extended wear and reliable data acquisition under ambulatory conditions.[172] Importantly, the integration of artificial intelligence (AI) and cloud-based analytics has improved feature extraction, noise suppression, and predictive modelling of cardiovascular events, strengthening the clinical value of wearable systems for preventive cardiology.[173] Recent studies highlight the growing adoption of smartwatches and multisensor wearables capable of identifying early warning signs of heart failure and structural cardiac abnormalities, reinforcing their translational potential for population-scale cardiovascular screening.[174,175] Market-level analyses also indicate rapid global growth in wearable cardiac devices, underscoring their expanding role in decentralized and preventive healthcare ecosystems.[176] Collectively, these developments position wearable sensors as complementary technologies to non-invasive cholesterol assessment, particularly for continuous cardiovascular risk monitoring and early disease detection.

Despite their promise, wearable platforms for non-invasive cholesterol monitoring face several critical practical barriers that have not yet been fully resolved. Sensor fouling represents one of the most significant challenges: electrochemical cholesterol sensors operating on sweat or saliva are continuously exposed to a complex biofluid matrix containing proteins, mucins, cellular debris, and inorganic salts. These species adsorb onto the electrode surface over time, progressively blocking the active sites of immobilised cholesterol oxidase and attenuating the electrochemical signal.[177] Calibration drift is

Table 14 Consolidated quantitative comparison of optical and non-optical methods

Method	Sensitivity	Selectivity	Detection limit	Cost	Sample	Portability potential	Real time	Clinical validation maturity[a]	Ref.
Diffuse reflectance spectroscopy	Moderate to high	Moderate	Ex vivo phantom contrast at ~25–200 mg dL^{-1} equivalent	Moderate	Skin	High	Yes	Low	96
Fluorescence spectroscopy	High	High	Skin-surface LOD not standardised; ~0.0004–0.02 mg dL^{-1} equivalent	Moderate	Skin	Moderate	Yes	Low	104
Photoacoustic spectroscopy	High	High	Tissue lipid contrast at \t{0.5–5 mg dL^{-1} (phantoms)}	High	Tissue	Moderate	Yes	Low	135
Raman spectroscopy	Very high	Very high	1–5 mg dL^{-1} (tissue/serum equivalent)	High	Tissue/ plaque/ serum	Low to Moderate	Yes	Low	150
Infrared spectroscopy	High	High	5–15 mg dL^{-1} in serum/tissue (ATR-FTIR)	Moderate to high	Tissue	Moderate	Yes	Low to Moderate	161
Surface plasmon resonance	Very high	Very high	0.1–10 nM (biosensor chip)	High	Sample contact	Low	Yes	Low	92
Electrochemical sensors	High	Moderate to high	0.02–0.19 mg dL^{-1}	Low	Saliva/ sweat	High	Yes	Moderate	58
Transdermal biosensors	Moderate	Moderate	0.04–0.39 mg dL^{-1}	Moderate	Interstitial fluid	Moderate to high	Yes	Low	64
Impedance spectroscopy	Moderate to high	Moderate	0.19–1.94 mg dL^{-1}	Moderate	Biofluids/ tissue	Moderate	Yes	Low	66
RF/microwave techniques	Moderate	Low to Moderate	No cholesterol-specific LOD established	High	Tissue	Moderate	Yes	Low	68
Ultrasound imaging	Moderate	Moderate	0.02–0.05 mm	High	Carotid artery/ tissue	Moderate	Yes	Moderate	165
MRI/MRS	Very high	Very high	Hepatic fat LOD 1–2% (MRI-PDFF) CE-MRS 0.5 mmol L^{-1} eq	Very high	Tissue	Low	No	Moderate	82
NMR spectroscopy	Very high	Very high	0.1–0.5 mmol L^{-1} (serum lipoproteins)	Very high	Blood/ urine	Low	No	Moderate to high	73

[a] Clinical validation maturity classification criteria: ratings are assigned using a four-tier framework. Low: no published peer-reviewed clinical study in human participants; evidence limited only to proof-of-concept demonstrations, *in vitro* experiments, *ex vivo* tissue models, or computational simulations. No regulatory bodies submission exists for the specified cholesterol-relevant indication. Moderate: at least one published clinical study in human participants ($n \geq 20$) reporting method output against an accepted reference standard (*e.g.*, enzymatic serum lipid panel, validated imaging modality, or histopathology), or existing FDA 510(k) clearance/CE marking for a closely related cardiovascular or lipid-relevant indication. Moderate to high: multiple independent clinical validation studies across diverse populations, or a single large-scale validation study ($n > 100$), with regulatory clearance for a cholesterol-related diagnostic indication and demonstrated multi-site reproducibility. High: regulatory clearance as a primary cholesterol or lipoprotein measurement device with direct prospective validation against venous enzymatic assay reference standards across diverse populations and inclusion in clinical practice guidelines for lipid management.

a closely related problem: enzyme-based sensors are inherently susceptible to activity loss due to temperature fluctuations. Finally, real-world use requires proper attachment, cleaning, and regular replacement of sensing elements, and user adherence is often inconsistent. So far, no sweat-based wearable cholesterol device has been validated in large real-life clinical studies, which is still needed for clinical adoption.[178]

6.1. Global applicability and point-of-care diagnostics in low-resource settings

Cardiovascular diseases remain the leading cause of mortality worldwide, with a disproportionate burden observed in low- and middle-income countries (LMICs), where access to centralized laboratory diagnostics and specialist care is often limited.[179] In response, the World Health Organization (WHO) has emphasized the importance of decentralized point-of-care (POC) diagnostic strategies to strengthen cardiovascular disease prevention, screening, and long-term management at the primary healthcare and community levels.[180,181] WHO policy frameworks and essential diagnostics lists specifically advocate the deployment of portable, affordable, and user-friendly diagnostic technologies capable of operating outside conventional laboratory infrastructures.[182]

Wearable sensors and POC diagnostic devices align closely with the WHO-endorsed ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable), making them particularly suitable for resource-constrained environments.[183,184] Recent global health studies demonstrate that decentralized POC technologies can substantially improve early detection, treatment adherence, and longitudinal monitoring of cardiovascular risk factors by reducing the reliance on trained personnel and advanced infrastructure.[185,186] In addition, the integration of wearable and handheld diagnostics with mobile health platforms enables remote data transmission and clinical decision support, facilitating the delivery of scalable cardiovascular care in underserved populations.[187]

Consequently, the inclusion of wearable and POC diagnostic frameworks in non-invasive cholesterol monitoring research strengthens the global relevance of this review and aligns emerging technologies with WHO-recommended strategies for equitable, accessible, and sustainable management of cardiovascular disease worldwide.[188] It is important to consider whether non-invasive cholesterol technologies are affordable and practical for low-resource healthcare settings. Advanced optical systems such as OCT are expensive (typically USD 20 000–150 000), require stable power, skilled man power, and are therefore difficult to deploy in primary care centres in low- and middle-income countries.[189] MRI and MRS are even more costly and infrastructure-intensive, needing specialised facilities and trained personnel, making them unsuitable for routine community use. For decentralised screening as per the direction measures of WHO, future research should prioritise these affordable, easy-to-use technologies that require minimal training and function reliably in typical LMIC healthcare environments.

7. Challenges and future directions

This paper identifies several key challenges that persist in the field of cholesterol monitoring, particularly concerning sensitivity and specificity. One of the primary issues is the need for methods that can accurately detect low concentrations of cholesterol in various biological samples, such as saliva or interstitial fluid, where the levels may be significantly lower than in blood. This challenge is compounded by the presence of interfering substances that can affect the accuracy of measurements, leading to false positives or negatives.

Another significant challenge is the biological variability among individuals, which can influence cholesterol levels due to factors such as diet, genetics, and overall health. This variability necessitates the development of standardized protocols and calibration methods to ensure consistent and reliable results across different populations and settings. Additionally, many existing techniques may lack the robustness required for long-term monitoring, particularly in wearable devices that must function effectively under varying environmental conditions.

To address these challenges, this study proposes several potential research directions aimed at enhancing the reliability and effectiveness of cholesterol monitoring techniques. One promising avenue is the integration of advanced materials, such as nanomaterials and biomimetic sensors, which could improve the sensitivity and specificity of detection methods. Research into the development of multifunctional biosensors that can simultaneously measure multiple biomarkers, including cholesterol, could also provide a more comprehensive view of an individual's metabolic health.

7.1. Clinical translation barriers (scalability, standardization, and regulatory pathways)

Addressing clinical translation barriers is essential for converting proof-of-concept demonstrations into deployable point-of-care or wearable cholesterol-related monitoring systems.

7.1.1. Scalability and manufacturability. PA systems often rely on precise alignment of optical excitation and acoustic detection, and consistent coupling is difficult to reproduce across low-cost portable platforms.[190] Scaling to manufacturable hardware requires robust component selection (light sources, transducers), thermal management, and mechanical stabilization to limit drift and inter-unit variability.[177]

7.1.2. Standardization of protocols and datasets. For both PA and microwave/RF, clinical generalization requires standardized acquisition protocols (probe pressure, coupling medium, target site definition, temperature control) and multi-site datasets. Lack of standardized ground truth definitions (*e.g.*, plaque lipid percentage *vs.* biochemical assays *vs.* imaging references) can lead to inconsistent performance reporting across studies.[178]

7.1.3. Regulatory approval and clinical validation. Clinical translation typically demands evidence of analytical validity (repeatability, accuracy), clinical validity (association with clinically relevant endpoints), and clinical utility. For PA devices targeting plaque composition, demonstrating impact on diagnostic decisions and outcomes is necessary. For any claim of "non-invasive cholesterol measurement," direct comparison against venous enzymatic lipid panels across diverse populations and conditions would be required, alongside robust quality systems and risk management consistent with medical device regulations.[191]

7.1.4. Real-world confounders and reproducibility. Motion artefacts, skin pigmentation, tissue thickness, perfusion changes, and temperature/humidity can degrade signal quality in both PA and microwave/RF. These effects must be mitigated through hardware design (stabilized coupling), signal processing, and model calibration strategies before point-of-care deployment is realistic.

Furthermore, the exploration of machine learning and artificial intelligence in data analysis could enhance the interpretation of cholesterol measurements, allowing for more accurate predictions and personalized health recommendations. Collaborative efforts between researchers, clinicians, and technology developers are essential to create user-friendly, cost-effective, and accurate monitoring systems that can be deployed in various healthcare settings.

7.2. AI/ML integration

In optical spectral pipelines, ML primarily helps in (i) denoising and baseline correction, (ii) feature extraction and dimensionality reduction, (iii) spectral unmixing and regression to estimate biomarkers or composition, and (iv) domain generalisation across instruments and subjects.[192] The most commonly used algorithms are

- *PLSR (Partial Least Squares Regression)*: it is widely used in spectroscopy because it handles collinearity and compresses spectral data to latent variables aligned with the target variable, improving stability with limited samples.[193]
- *SVR (SupportVector Regression)*: it is effective for nonlinear relationships and small-to-medium datasets, often improving prediction when the spectra–biomarker mapping is nonlinear.[194]

• *Neural networks (including shallow MLPs and deeper architectures)*: it can learn nonlinear mappings and interactions, but require careful regularization and larger, more diverse datasets to avoid overfitting.

A comparative spectroscopy-focused study reported excellent predictive performance for lipids including cholesterol using PLSR, SVR, and neural networks, supporting these as reasonable algorithm families to highlight when discussing spectral cholesterol prediction pipelines. In the context of non-invasive cholesterol detection, these machine learning algorithms address several key limitations inherent to optical spectroscopy of skin and tissue. First, cholesterol spectral signatures are weak and strongly overlapped with signals from other lipids, proteins, and water. Dimensionality reduction and latent-variable approaches such as PLSR help isolate cholesterol related spectral variance by projecting highly collinear spectral features into a smaller set of components maximally correlated with cholesterol concentration, effectively performing spectral unmixing and suppressing background interference. SVR and neural networks further improve this separation when spectral concentration relationships become nonlinear due to tissue scattering or heterogeneous lipid distribution. Second, substantial biological variability exists across individuals, arising from differences in skin thickness, pigmentation, hydration, lipid composition, and probe tissue coupling. Multivariate ML calibration models learn these inter subject variations from training datasets and map them to consistent cholesterol estimates, thereby normalising physiological variability that cannot be corrected by physical calibration alone. In comparative terms, PCA primarily serves as a preprocessing and feature-extraction method that reduces noise and spectral redundancy but does not itself provide quantitative prediction. PLSR is the most widely adopted regression approach in optical biospectroscopy because it compresses highly collinear spectral variables into latent components optimally correlated with cholesterol concentration, yielding stable and interpretable calibration with limited datasets. SVR extends this capability by modelling nonlinear relationships between spectral features and cholesterol levels, often improving prediction accuracy in heterogeneous tissue measurements, although it requires careful parameter tuning and larger training data to avoid overfitting. Neural-network models further enhance nonlinear mapping and interaction learning across complex spectral patterns, but their performance is strongly dependent on dataset size, diversity, and regularisation to ensure generalisation. Thus, PCA enhances spectral quality, PLSR provides robust linear quantitative calibration, SVR improves nonlinear prediction, and neural networks offer the highest modelling flexibility, indicating complementary functional roles within non-invasive cholesterol spectroscopy pipelines.

Finally, this paper emphasizes the importance of regulatory approval and clinical validation for new technologies, ensuring that they meet the necessary standards for safety and efficacy before widespread adoption. By addressing these challenges and pursuing innovative research directions, the field of cholesterol monitoring can move towards developing more effective tools for early disease detection and prevention, ultimately improving patient outcomes in cardiovascular health.

8. Conclusion

Non-invasive optical techniques for cholesterol quantification have emerged as a promising alternative to traditional invasive methods. Numerous approaches have been studied for the NI detection of cholesterol, while significant improvements in accuracy, precision, and dependability are still required to achieve the stringent standards to obtain regulatory clearance and successful commercialization of the device. This review provides a critical overview of optical techniques for truly non-invasive cholesterol assessment, alongside minimally invasive approaches that support point-of-care monitoring encompassing their underlying principles, advantages, limitations, and performance metrics based on wavelength. Despite these techniques having great potential, they encounter challenges related to sensitivity, long-term stability, specificity, biological variability, and calibration accuracy which can impact their overall effectiveness and reliability. Overcoming these obstacles is critical for creating reliable, clinically useful non-invasive cholesterol monitoring systems. Based on our comprehensive review, Compared to other techniques, we have proposed a viable technique for non-invasive cholesterol detection using IR spectroscopy. The absorption peaks of synthetic cholesterol should closely correspond to those of natural cholesterol, particularly in regions associated with hydroxyl (–OH), C–H, and C–O bonds, and it confirms that the synthetic model effectively mimics real cholesterol, making it suitable for testing purposes. Moreover, we conducted experiments utilizing Fourier Transform Infrared Spectroscopy on synthetic cholesterol samples. The results demonstrated the efficacy of our proposed non-invasive approach, which encompasses the performance falling under the acceptable range of cholesterol measurement. Future progress in non-invasive cholesterol monitoring is expected to rely on hybrid sensing architectures, multi-wavelength spectroscopy, and machine-learning-assisted signal interpretation to overcome biological variability.

Conflicts of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

This review article is entirely based on previously published and publicly available literature. All data analyzed, interpreted, and discussed in this study were extracted from peer-reviewed journal articles and authoritative databases cited throughout the manuscript. The authors did not generate or collect any new datasets as part of this work. Where applicable, relevant data sources and references have been clearly mentioned to allow verification and further exploration. Readers interested in accessing the underlying studies can refer to the reference list provided at the end of the manuscript for detailed bibliographic information.

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