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Infrared Microscopy: A Multidisciplinary Review of Techniques, Applications, and Ethical Dimensions

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ABSTRACT

Infrared microscopy has become a significant analytical technique with a transformative impact on various scientific disciplines. This review examines its applications in biomedical research, materials science, environmental monitoring, and art conservation. The non-invasive and label-free technique has revolutionized disease diagnostics, drug discovery, and tissue engineering by providing comprehensive molecular and cellular insights. In materials science, it has significantly advanced understanding of microstructure and material properties, facilitating the development of novel materials. In environmental monitoring, infrared microscopy plays a crucial role in assessing microplastics and atmospheric pollutants, supporting environmental protection efforts. In art preservation, the technique offers valuable insights into the composition and deterioration of historical artworks. Recent advancements in sensor technology, particularly InGaAs and graphene-based detectors, coupled with artificial intelligence and machine learning, have greatly enhanced image analysis capabilities. The review identifies key challenges such as surpassing the diffraction limit and interpreting complex data. Ethical concerns, including data privacy and equitable access to technology, are also emphasized. Infrared microscopy remains a vital tool for advancing scientific knowledge and practical applications. Its impact is poised to expand with future technological developments, contingent upon addressing both technological challenges and ethical considerations.

Keywords: Infrared Microscopy Techniques; Sample Preparation; Multidisciplinary Applications; Ethical Considerations; Machine Learning and AI Techniques.

INTRODUCTION

Introduction to Infrared Imaging

Infrared (IR) imaging offers a unique opportunity to explore hidden aspects of the universe beyond human vision. In recent years, this advanced technology has made significant progress, providing valuable insights across biomedical science, environmental research, art conservation, and industrial applications. This article explores the fundamental principles of infrared imaging to give readers a comprehensive understanding of this intriguing field, as depicted in Figure 1.

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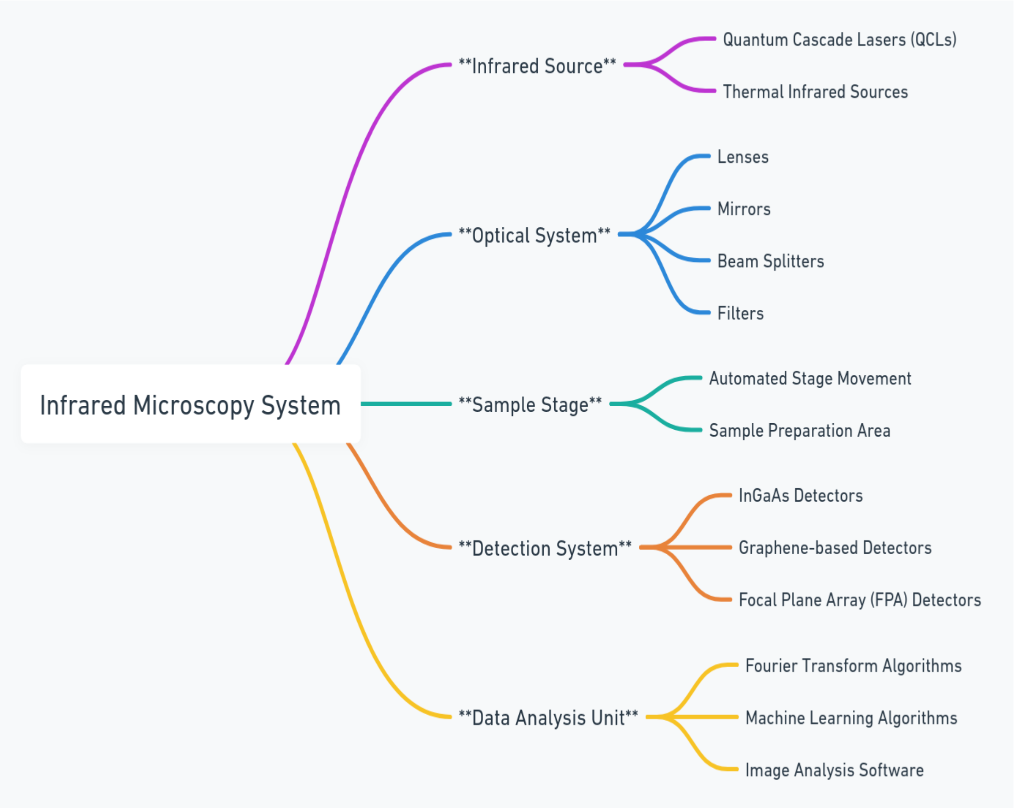

Figure 1 The essential elements of an infrared microscopy system. The diagram illustrates the main components of the system, such as the Infrared Source, Optical System, Sample Stage, Detection System, and Data Analysis Unit, along with their respective subcomponents.

The potential for significant advancements in both science and technology makes infrared imaging an area of great interest. The electromagnetic spectrum encompasses a wide range of wavelengths, from visible light to ultraviolet radiation and infrared rays. Specifically, the infrared portion extends from 700 nanometers (nm) to 1 millimeter (mm) [1]. Infrared radiation is categorized into three groups: near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR) [2], each with unique characteristics offering specific advantages in various fields. IR imaging systems detect infrared radiation emitted or reflected by objects and convert it into electrical signals [3]. These signals are processed to generate intricate thermal maps that reveal details beyond human vision [4]. Traditional IR imaging techniques commonly use specialized sensors and thermal cameras [5]. The ability to convert these signals into visual images provides significant insights into the environment, revealing previously unidentified natural phenomena and enabling new avenues of exploration and understanding. The integration of conventional microscopy with infrared spectroscopy in infrared microscopy has transformed scientific research by providing unparalleled spatial resolution and chemical specificity at the microscale. This non-invasive, contactless technology offers detailed information about materials without causing harm or alteration [6]. Its versatility allows adaptation to various environments and experimental setups, fostering new opportunities for exploration. This review examines cutting-edge techniques that have propelled infrared microscopy to the forefront of discovery and explores the applications enabled by these advances. It also addresses the ethical implications and future prospects of infrared imaging technologies in this rapidly progressing field. By unveiling invisible phenomena in science, these methodologies enhance our understanding and revolutionize our approach to research and innovation.

The Emergence of Infrared Microscopy: A Brief History

The origins of infrared microscopy can be attributed to Sir Frederick William Herschel, a British astronomer and musician. In 1800, Herschel made the remarkable discovery of previously unseen radiation within the electromagnetic spectrum [7]. His experiments revealed a previously unidentified energy source, sparking further investigation in this emerging area of research. In the early 20th century, significant progress was made in thermal detection, specifically with thermopiles and bolometers. These advances paved the way for initial endeavors in visualizing infrared radiation [8]. The development of Fourier transform infrared (FTIR) spectroscopy in the mid-20th century marked a significant milestone in the advancement of FTIR microscopy. This technique allowed researchers to achieve unprecedented precision in probing chemical composition and molecular structure. Herschel's serendipitous discovery paved the way for significant technological advances, particularly in infrared microscopy. As a result, we are now in a modern era characterized by rapid technological progress and a wide range of applications for this microscopy technique.

The 21st century has brought about a new era in infrared microscopy. Recent advancements in techniques and technologies have significantly expanded the boundaries of achievable outcomes, enabling spatial resolutions and acquisition speeds that were previously unattainable. The integration of quantum cascade lasers (QCLs) and near-field microscopes has transformed the field, leveraging advanced computational techniques and machine learning algorithms to provide an extraordinary scientific experience [9].

Principles and Fundamentals of Infrared Microscopy

Infrared microscopy has revolutionized scientific exploration by enabling investigation into the previously unobservable realm of infrared radiation. To fully grasp the capabilities and possibilities of this phenomenon, it is crucial to understand the underlying principles that govern its functioning. Infrared microscopy operates by identifying unique absorption patterns exhibited by molecules within a sample, thereby providing insights into their chemical composition and structural characteristics [10]. A comprehensive understanding of optical microscopy and infrared spectroscopy is necessary to comprehend this process. Spectroscopy reveals different absorption patterns, known as molecular "fingerprints," arising from the unique energy levels present in different molecules [11]. Through spectral analysis, researchers gain valuable insights into the molecular composition of sample materials and develop an accurate understanding of their characteristics.

Optical microscopes utilize visible light and magnifying lenses to produce enlarged images. However, their resolution is constrained by diffraction, limiting them to approximately half the wavelength of the light used, often falling within the range of invisible light [12]. Specialized optics can overcome this limitation, enabling higher-resolution imaging and more advanced chemical analysis capabilities associated with infrared microscopy techniques. Integration of infrared microscopy has significantly transformed microscale imaging and chemical analysis by combining two robust techniques into a unified platform [13]. This approach utilizes modern Fourier transform infrared (FTIR) spectroscopy to provide enhanced spectral resolution, improved signal-to-noise ratio, and faster data acquisition. The tunable nature of Quantum Cascade Laser (QCL)-based systems and near-field infrared microscopy has led to significant advancements in achieving exceptional levels of detail and specificity.

Researchers employ various sampling methods, such as transmission, reflection, or attenuated total reflectance

(ATR), to analyze different sample types under varying conditions. Advanced computational algorithms, including multivariate analysis and machine learning, efficiently perform complex data analyses and extract valuable information rapidly [14]. This text provides an in-depth overview of the fundamentals of infrared imaging technology, potential applications (as shown in Table 1), and its transformative impact in scientific research.

Table 1. A comparative analysis of various infrared microscopy techniques, focusing on their scientific principles, advantages, limitations, and key references.

Technique	Principle	Advantages	Limitations	Reference
Raman Infrared Microscopy	Inelastic scattering of light by sample molecules; shift in frequency provides molecular information	Label-free; Non-destructive; Complementary to IR microscopy	Limited sensitivity; Fluorescence interference	[15]
Thermal Infrared Microscopy	IR detection of temperature differences in the sample due to its thermal properties	Non-contact; Non-destructive; Useful for material analysis	Limited to samples with temperature variations	[16, 17]
Fourier Transform Infrared (FTIR) Microscopy	Interferometry and Fourier Transform to obtain IR spectra	High resolution; Rapid data acquisition; High sensitivity	Requires precise alignment and calibration	[18]
Quantum Cascade Laser-based Infrared Microscopy	Tunable laser sources for the IR spectral region; narrow linewidth	High spatial and spectral resolution; Fast acquisition	Limited to a specific wavelength range	[19]
Near-field Infrared Microscopy	Near-field scanning optical microscopy with IR light source	Sub-wavelength spatial resolution; Non-destructive	complex setup; Slow imaging process	[20]
InGaAs Detector-based Microscopy	Indium Gallium Arsenide detectors for NIR region	High sensitivity; Fast response time	Limited to the NIR range	[21]
Graphene-based Detectors	Graphene's unique electronic properties for IR detection	Broadband detection; Ultrafast response; High sensitivity	Requires advanced fabrication techniques	[22]
Transmission Infrared Microscopy	The transmitted IR light passes through the sample and is detected	Useful for thin samples; Good for biological applications	Limited to transparent samples	[23]
Reflection Infrared Microscopy	Reflected IR light from the sample surface is detected	Suitable for opaque samples; Non-destructive	Limited to surface analysis; Possible specular reflections	[24]
Attenuated Total Reflection (ATR) Infrared Microscopy	Sample in contact with ATR crystal; evanescent wave interacts with the sample	Minimal sample preparation; Suitable for most samples	Limited penetration depth; Requires good sample contact	[25]
Hyperspectral Infrared Microscopy	Combines IR microscopy with hyperspectral imaging for detailed spectral and spatial information	High spectral and spatial resolution; Rich data sets	Longer acquisition times; Requires advanced data processing	[26]
Time-resolved Infrared Microscopy	Time-gated detection of transient IR signals	Studying fast chemical reactions or transient phenomena	Requires specialized equipment; Limited temporal resolution	
Polarization-sensitive Infrared Microscopy	Analyzing Polarization-Dependent Response of the sample to IR light	Provides insight into the sample's anisotropy and orientation	Limited to samples with anisotropic properties	[27]

Fourier Transform Infrared (FTIR) Microscopy: Advancements in Spectral Imaging and Analytical Capabilities

FTIR microscopy transforms scientific observation of samples by providing exceptional chemical specificity at the microscale. FTIR combines advanced spectral analysis with optical microscopy to efficiently capture high-resolution infrared spectra in significantly less time compared to conventional techniques. The fundamental aspect of this method is Fourier transform; a mathematical technique used to analyze interferograms acquired from an interferometer. It effectively converts these interferograms into high-resolution infrared absorption spectra.

FTIR microscopy has revolutionized interdisciplinary research, spanning materials science and biomedical imaging. It offers notable advantages over alternative spectroscopic techniques, primarily due to its swift data acquisition and remarkable signal-to-noise ratio achieved through Fellgett's Advantage or multiplex advantage [28]. To enhance these benefits, advanced sampling modes such as transmission, reflection, and attenuated total reflectance (ATR) have been developed. These modes are suitable for various sample types and experimental conditions. The ATR mode, in particular, enables surface probing of challenging samples that are not suitable for transmission measurements due to the internal reflection element [29].

Incorporation of focal plane array (FPA) detectors into Fourier transform infrared (FTIR) microscopy systems has significantly enhanced the technique's capabilities [5]. These detectors enable researchers to efficiently generate high-resolution chemical maps over large areas by collecting extensive spectral data from thousands of spatial points simultaneously. With contemporary computational techniques and advanced hardware advancements like FPA detectors [19], FTIR microscopy is leading the field of spectral imaging, enabling scientists to address complex issues and gain unprecedented insights.

Multivariate analysis techniques, such as principal component analysis (PCA) and partial least squares (PLS) regression, effectively extract valuable information from complex datasets. Additionally, machine learning algorithms proficiently identify subtle features and construct predictive models using FTIR data [30].

Comparative Analysis of Mapping and Imaging Modalities in Fourier Transform Infrared (FT-IR) Microscopy

Fourier Transform Infrared (FT-IR) microscopy utilizes two main modalities: mapping and imaging. Although they are often used interchangeably, these terms refer to separate techniques in the acquisition and visualization of spectral data [31]. Mapping in FT-IR involves collecting spectra at defined points across the sample [32]. Typically, the stage holding the sample moves in an X-Y grid pattern, and a spectrum is recorded at each point. This results in a series of individual spectra, which can be processed and compiled into a map representing specific chemical information. FT-IR mapping is highly versatile, allowing for high-resolution analysis; however, it can be time-consuming, especially for large areas [33].

In contrast, FT-IR imaging involves the simultaneous collection of spectral data over a wide area of the sample [34]. Using a focal plane array (FPA) detector, it captures spatial information in two dimensions along with the spectral dimension [35]. Therefore, FT-IR imaging is often faster and more suited for analyzing larger samples or providing a general overview of the sample composition. However, it may have lower spectral resolution compared to mapping [36]. In practice, the choice between mapping and imaging depends on the specific requirements of the analysis. Mapping is preferred when pinpoint spectral accuracy and high spatial resolution are paramount, such as in the analysis of microstructures or small inclusions within materials. Imaging, on the other hand, may be chosen for a more comprehensive, rapid assessment of larger areas or when contextual information is crucial.

In summary, FT-IR mapping excels at detailed, high-resolution chemical characterization at specific points [37], while FT-IR imaging provides a broader view by simultaneously capturing spectral and spatial information across an extended area [38]. Both modalities are invaluable tools in infrared microscopy, and their judicious application can yield rich insights into the molecular and structural attributes of samples across diverse fields.

Quantum cascade laser-based infrared microscopy

Quantum Cascade Lasers (QCLs) have significantly transformed infrared microscopy by introducing unparalleled performance and functionality. Leveraging intersubband transitions in quantum wells to produce tunable, coherent radiation across mid- and far-infrared regions, these semiconductor lasers offer numerous advantages for scientific research [39]. QCL-based infrared microscopy enables rapid data acquisition with improved signal-to-noise ratios, allowing real-time, in-situ measurements [40]. This capability is supported by their broad tunability and high spectral brightness, surpassing existing technologies.

Advanced detection techniques such as Focal Plane Arrays (FPAs) benefit from QCLs' increased brightness, providing researchers with hyperspectral images and exceptional spatial resolution in record times [41]. One notable advancement is Quantum Cascade Laser-based Near-Field Scanning Optical Microscopy (QCL NSOM), combining QCLs and near-field scanning optical microscopy (NSOM). This technique surpasses the diffraction limit, achieving resolutions in the tens of nanometers range. It opens novel opportunities in nanoscale material characterization, particularly in plasmonics, phonon polaritons, and molecular vibrations, offering unprecedented detail.

QCL-based infrared microscopy has significantly impacted various scientific disciplines, including materials science, environmental monitoring, and biomedical imaging [43, 44]. Its broad applications facilitate the investigation of semiconductor materials and precise analysis of defects in two-dimensional materials, as well as the precise mapping of chemical composition within biological tissues.

Near-field infrared microscopy

Consider a hypothetical scenario where the diffraction limit no longer restricts observation, enabling us to explore the intricate and enigmatic nanoscale. Near-field infrared microscopy (NFIM) has overcome this barrier, providing unparalleled insights into nanomaterials [45]. This innovative method holds immense potential for scientific investigation, inspiring wonder with its capability to reveal previously inconceivable information. The fundamental concept integrates near-field scanning optical microscopy (NSOM) principles with infrared spectroscopy, generating evanescent fields that are probed using specific methods such as aperture probes or scattering-type scanning near-field optical microscopes (s-SNOM). These fields are then transformed into propagating waves, producing high-resolution images with spatial resolutions as low as tens of nanometers.

NFIM's innovative technology offers unprecedented access to data on chemical composition, structural characteristics, and vibrational information at the nanoscale [46], providing significant insights into material behavior in nano-environments. This capability allows researchers to understand the dynamic nature of our universe and discover transformative applications. The term "near-field" refers to the region in close proximity to an object or source of interest.

Infrared microscopy has profoundly influenced various fields, including two-dimensional materials, plasmonic nanostructures, the study of biological systems, and molecular assemblies [47, 48]. The field continues to advance with the introduction of quantum cascade lasers (QCLs), which offer enhanced brightness and rapid tunability [49], along with novel probe designs and fabrication methods enabling higher-resolution imaging and simultaneous measurement of multiple sample properties [50].

Advancements in Sensor Technologies: A Comparative Study of InGaAs and Graphene-Based Detectors in Future Applications

InGaAs detectors are widely recognized as the preferred choice for near-infrared spectroscopy and imaging due to their high quantum efficiency, minimal dark current, and excellent temperature stability [51]. The introduction of graphene-based sensors has significantly enhanced the performance and sensitivity of spectral imaging, sparking a revolution in this field. This article aims to comprehensively explain InGaAs and graphene-based detectors, aiming to pique curiosity about the potential capabilities of these innovative sensors.

Advancements such as the adoption of large-format focal plane arrays (FPAs) and high-speed cameras based on InGaAs technology have expanded the application range of these detectors in fields such as telecommunications, environmental monitoring, and biomedical imaging [52]. Graphene-based sensors are particularly transformative in infrared detection, surpassing the capabilities of InGaAs detectors. Graphene, composed of a single layer of carbon atoms arranged in a hexagonal lattice, exhibits unique electronic and optical properties that make it highly suitable for advanced sensor applications [53, 54].

Graphene-based detectors offer several advantages over traditional InGaAs detectors, including a broader spectral response, enhanced responsivity, and rapid response times. Moreover, their compatibility with Complementary Metal-Oxide-Semiconductor (CMOS) fabrication processes enables cost-effective production of sensor arrays on a large scale [55]!

Exploiting Infrared Imaging CCD Cameras: Transforming Sensitivity and Resolution

The integration of charge-coupled device (CCD) cameras has revolutionized infrared (IR) imaging, enabling the capture of high-resolution images across the infrared spectrum [56]. These cameras convert light into electronic signals interpretable as pictures [57]. CCDs designed for infrared imaging exhibit remarkable sensitivity to IR radiation [58], facilitated by specialized sensors and cooling mechanisms that enhance detection accuracy of faint IR signals. Enhanced spatial resolution is another significant benefit [59]; each photodetector corresponds to a pixel on the image, with higher-end models featuring densely packed arrays of detectors for sharper visuals, making them particularly suitable for detailed material science studies at microscopic levels.

Excellent quantum efficiency further enhances their advantages [60, 61]; photons are efficiently converted into electrons, resulting in images with enhanced contrast and dynamic range, attributable to reduced internal camera noise levels [62]. Moreover, these devices can integrate seamlessly with various optical components or filters to meet specific application needs; for instance, biomedical imaging may require specific wavelengths to enhance visibility of certain cellular components over others [63].

Multispectral and Hyperspectral Imaging

Multispectral photography captures images using various spectral bands, enabling simultaneous analysis across multiple wavelengths and providing an enhanced perspective on reality [64]. This technology finds extensive application in remote sensing for agriculture, environmental monitoring, and cultural heritage conservation efforts. Multispectral imagery allows researchers to uncover crucial information regarding materials' composition, structures, and functional properties.

Hyperspectral imaging offers precise spectral signatures from a wide range of contiguous bands [65]. Advances in sensor technologies such as InGaAs and graphene detectors have led to the development of compact yet highly efficient imaging systems capable of capturing detailed spectral information at high resolutions across a broad range of wavelengths. Machine learning algorithms, along with deep learning techniques, have expanded capabilities in the analysis of multispectral and hyperspectral data [66].

Computational Approaches in Image Analysis: Integrating Machine Learning and Artificial Intelligence for Advanced Data Interpretation

The application of advanced artificial intelligence (AI) and machine learning techniques has significantly transformed the field of infrared imaging through the analysis of multispectral and hyperspectral images. Computational methods such as k-means clustering, hierarchical clustering, support vector machines, and random forests provide efficient means to extract valuable insights from complex image data [67, 68].

Deep learning, a subfield of machine learning, enables the creation of sophisticated artificial neural networks capable of capturing intricate patterns and abstractions in datasets. Convolutional neural networks (CNNs), in particular, have gained popularity and demonstrated efficacy in various image analysis tasks, including object recognition, segmentation, and classification [69, 70]. Advanced neural network models like Residual Networks (ResNets) and U-Nets have significantly advanced infrared image processing [71, 72]. ResNets address the issue of vanishing gradients through deep layers and skip connections, enabling comprehensive analysis of intricate image features [73]. U-Nets have shown exceptional performance in segmenting infrared images, crucial for applications such as thermal fault detection, due to their unique encoder-decoder architecture [74].

The integration of multispectral imaging techniques and advanced computational methods, such as AI and ML, has expanded the capabilities of infrared imaging. This combination allows for the detection and analysis of complex spectral signatures and subtle variations in material composition or functional properties [27].

Domain adaptation techniques within transfer learning frameworks have become pivotal in IR imaging, addressing challenges posed by domain shift when models trained on visible spectrum images are adapted to perform accurately on infrared spectrum images. This recalibration is crucial in applications like cross-spectral medical diagnostics, where models trained on extensive RGB image datasets need adjustments for interpreting IR images [75]. For instance, models originally trained on the ImageNet dataset have been successfully adapted for thermal image analysis [76, 77], enhancing early detection of skin diseases and reducing reliance on extensive thermal datasets.

Generative Adversarial Networks (GANs) have significantly improved the quality of infrared images. Super-resolution GANs (SRGANs) are employed to enhance the resolution of low-resolution infrared images, a common challenge in thermal imaging due to sensor limitations [78, 79]. SRGANs use adversarial training to generate high-resolution images that retain crucial thermal details essential for accurate analysis [80]. This technology has been applied extensively in urban thermal imaging [81, 82], making significant contributions to heat mapping and energy efficiency research.

The emerging field of infrared (IR) imaging holds immense potential for advancements across various disciplines, as highlighted in Table 2, including environmental monitoring and medical diagnostics.

Elucidating Molecular Structures via Advanced Sample Preparation Techniques in Infrared Microscopy

Sample preparation is crucial for successful infrared microscopy, ensuring optimal imaging conditions and maximizing data quality [91]. To achieve maximum light transmission through the sample, thin sections must be created using techniques such as microtoming, cryo-sectioning, or focused ion beam milling [92]. These methods are adaptable for various sample types and are essential for obtaining precise spectral data while minimizing artifacts introduced by the microscope.

Researchers can optimize experimental performance and obtain meaningful results by tailoring their preparation strategies based on an understanding of how infrared light interacts with samples. This approach ensures dependable data acquisition, leading to successful outcomes in infrared microscopy.

Sample mounting plays a critical role in obtaining accurate and precise imaging results [93]. Scientists have developed several mounting methods, including the KBr pellet technique, reflective methods, and attenuated total reflectance (ATR), each suitable for different sample types and infrared imaging modes. Each method offers distinct advantages and presents various challenges depending on the properties of the sample being imaged and the desired outcomes (as summarized in Table 3).

Table 2. A comparative summary of machine learning and artificial intelligence techniques used in infrared microscopy, detailing their applications, advantages, limitations, and key references.

Method	Application in Infrared Microscopy	Advantages	Limitations	Reference
Independent Component Analysis (ICA)	Blind source separation and decomposition of IR microscopy data	Identifies statistically independent components	Assumes statistical independence; Sensitive to noise	[83]
Supervised Learning	Image classification, segmentation, and identification of features in IR microscopy data	High accuracy; Robustness to noise; Human-interpretability	Requires labeled data; Can overfit if training data is small	[84]
Unsupervised Learning	Clustering and dimensionality reduction of IR microscopy data	No labeled data required; Can uncover hidden patterns	Less interpretable; Sensitive to initial conditions	[85]
Convolutional Neural Networks (CNNs)	Feature extraction and image analysis in IR microscopy data	Automatic feature extraction; High accuracy	Require large amounts of data; Computationally expensive	[86]
Support Vector Machines (SVMs)	Classification of IR microscopy data	Robust to outliers; Can handle high-dimensional data	Requires parameter tuning; Not efficient for large datasets	[87]
Principal Component Analysis (PCA)	Dimensionality Reduction and visualization of IR microscopy data	Reduces data complexity; Retains essential information	Assumes linear relationships; Can be sensitive to noise	[85]
Transfer Learning	Applying pre-trained models to IR microscopy data analysis	Accelerates training time; Reduces data requirements	May not be ideal for highly specific tasks	[84]
Reinforcement Learning	Optimizing sample positioning and data acquisition in IR microscopy experiments	Adapts to new situations; can optimize for multiple objectives	Requires careful reward function design; Can be slow to converge	[88]
Generative Adversarial Networks (GANs)	Synthesizing Realistic Infrared Microscopy Data for training and evaluation	Generates high-quality data; Can improve model performance	Can be difficult to train; Sensitive to model architecture	[89]
Autoencoders	Denoising, compression, and feature extraction of IR microscopy data	Can reduce noise and data complexity	May not retain all relevant information	[90]
Deep Learning	Advanced feature extraction and classification of IR microscopy data	Can model complex relationships; High accuracy	Requires large amounts of data; Can be difficult to interpret	[27]

For deeper insights into the molecular composition or structure of a sample, additional techniques involving chemical or enzymatic modifications, functionalization, or isotopic labeling can be employed.

Table 3. A systematic comparison of sample preparation techniques in infrared microscopy, highlighting their applications, advantages, limitations, and key references. The table aims to guide researchers in selecting methods aligned with their experimental needs and to provide a comprehensive understanding of the diverse techniques.

Technique	Application in Infrared Microscopy	Advantages	Limitations	Reference
Cryo-sectioning	Preserving hydrated samples for IR microscopy	Maintains sample integrity; Reduces thermal degradation	Requires cryocapable equipment; Freezing artifacts	[94]
KBr pellet technique	Creating transparent, homogeneous samples for IR microscopy	Simple preparation; Good for solid and powdered samples	Requires large sample amount; Limited sensitivity	[95-97]
ATR (Attenuated Total Reflectance)	Minimizing sample preparation for IR microscopy	Minimal sample preparation; Works with various sample types	Limited penetration depth; Requires ATR accessory	[98]
Microtoming	Slicing samples into ultra-thin sections for IR microscopy	Enables high-resolution imaging; Reduces sample distortion	Requires specialized equipment; Skilled handling	[99]
Embedding	Stabilizing Samples in a solid matrix for IR microscopy	Preserves sample integrity; Facilitates sectioning	Possible matrix interference: Matrix selection is critical	[100]
Drop-casting	Depositing Liquid Samples on a substrate for IR microscopy	Simple preparation; Suitable for liquid samples	Requires controlled drying; Potential for uneven deposition	[101]
Air-drying/spin-coating	The formation of thin films of liquid samples for IR microscopy	Fast sample preparation; Good for liquid samples	Requires equipment; Can introduce artifacts	[102]
Liquid cells	Analyzing liquid samples in situ using IR microscopy	Enables in situ analysis; Minimizes sample disturbance	Requires specialized equipment; Limited spatial resolution	[103]
Thin sectioning	Preparation of thin, uniform samples for IR microscopy	Enables high spatial resolution; Minimizes scattering	Requires skilled handling; Time-consuming	[104]

Addressing Technical Challenges in Infrared Microscopy for Innovations in Analytical Excellence

Infrared microscopy provides a robust method for analyzing the molecular structure and composition of various materials with enhanced precision [105]. Despite its numerous advantages, researchers face specific challenges that can hinder their progress, as detailed in Table 4. This review aims to clarify the complexities associated with infrared imaging methods and offer recommendations for effectively addressing them to optimize their potential.

One significant challenge is the absorption of infrared radiation by water molecules, which can attenuate signals or obscure spectral features in examined samples [106]. To mitigate this issue, maintaining optimal humidity levels during experiments and adhering to rigorous sample preparation protocols are crucial. Additionally, mathematical algorithms can be applied to correct distortions caused by water absorption [107].

Another limitation is the diffraction limit of infrared microscopy, which researchers have overcome using near-field techniques such as super-resolution imaging technologies [108].

Addressing the challenge of low signal-to-noise ratio (SNR) in infrared microscopy data acquisition involves implementing various strategies. These include optimizing microscope configurations, using advanced detectors and signal processing techniques, and applying noise reduction algorithms alongside spectral averaging [109].

Table 4. A comprehensive examination of primary obstacles in infrared microscopy, presenting practical resolutions alongside their respective benefits and constraints. This study addresses a range of topics, including light scattering and data acquisition time, by systematically evaluating the advantages and disadvantages of various solutions. The analysis is supported by relevant references. The objective is to offer a detailed analysis of the difficulties and potential solutions in the field.

Challenge	Solution(s)	Advantages	Limitations	Reference
Light scattering by the sample	Thin sectioning; ATR (Attenuated Total Reflectance); Focal plane array (FPA) detector	Improved signal-to-noise ratio; Enhanced resolution	Sample preparation; Equipment requirements	[110]
Low spatial resolution due to diffraction limit	Near-field infrared microscopy; super-resolution techniques; Confocal infrared microscopy	Higher spatial resolution; Better imaging capabilities	Complex techniques; Specialized equipment	[111]
Incomplete sample coverage	Hyperspectral imaging; Automated stage movement; Stitching algorithms	Comprehensive sample analysis; Improved data quality	Data processing; Instrumentation requirements	[112]
Challenges in data analysis	Machine learning and AI algorithms for image analysis; Hyperspectral unmixing algorithms; Spectral libraries	Improved data interpretation; Enhanced analysis speed	Algorithm development; Computational requirements	[113, 114]
Variability in sample composition	Multivariate analysis; Principal component analysis (PCA); Partial least squares (PLS) regression	Better understanding of complex samples; Improved quantification	Requires extensive computational analysis	[115]
Weak or overlapping infrared signals	Subtraction techniques; Multivariate curve resolution; Two-dimensional correlation spectroscopy	Improved spectral resolution; Clearer identification of signals	Requires advanced data processing techniques	[116, 117]
Sample fluorescence interference	Time-gated detection; Fluorescence quenching agents	Reduced fluorescence interference; Improved spectral quality	Additional sample preparation; Equipment requirements	[118]
Non-uniform sample thickness	Automated focus adjustment; Depth profiling	Enhanced image quality; Accurate measurements	Additional equipment requirements; Increased analysis time	[119]
Limited sensitivity	Enhanced sensor technologies (InGaAs, graphene-based detectors); Signal amplification methods	Increased sensitivity; Improved detection capability	Equipment costs; Complexity	[120]
Sample damage due to high-energy radiation	Cryo-sectioning; Rapid data acquisition techniques; Minimizing exposure time	Preservation of sample integrity; Reduced damage	Specialized equipment; Sample preparation	[121]
Long data acquisition times	Fourier transform infrared (FTIR) microscopy; Quantum cascade laser (QCL) microscopy; FPA detectors	Faster data acquisition; Higher throughput	Equipment costs; Complexity	[41]

Applications of Infrared Microscopy Across Disciplines

Elucidating Molecular Mechanisms in Biomedical and Life Sciences via Infrared Microscopy

Infrared microscopy has significantly transformed the fields of biomedical and life sciences by offering insights into the molecular complexities of biological systems [122]. This technology advances disease diagnostics and cellular imaging through its label-free [123], non-invasive [124] approach to sample analysis. Its transformative capabilities have a profound impact on patient care, providing precise diagnoses for conditions such as cancer, neurodegenerative disorders, and infectious diseases in a swift and minimally invasive manner.

Real-time visualization of cellular components and exploration of dynamic molecular processes are facilitated by infrared microscopy [125]. In drug discovery and development, this powerful tool aids in target recognition, monitors cellular responses to drugs, evaluates safety profiles, and assesses the efficacy of therapeutic compounds, thereby advancing the development of effective therapies [88].

Infrared microscopy also plays a crucial role in tissue engineering and regenerative medicine [126]. Its nondestructive and label-free imaging capabilities reveal components of cellular and extracellular matrices essential for developing innovative tissue scaffolds [127], optimizing stem cell differentiation protocols [128], and monitoring the integration of engineered tissues within host organisms [129], thereby advancing personalized regenerative therapies.

Similarly, infrared microscopy serves environmental and plant sciences by enabling researchers to explore the molecular composition of environmental samples [110], assess the effects of pollutants on natural systems [130], and understand the molecular mechanisms involved in plant growth [131], development, and responses to stressors.

Infrared microscopy presents numerous possibilities across various fields, as highlighted in Table 5.

Material Science: Investigating the Microstructure of Materials

Infrared microscopy has significantly enhanced the field of material science by providing insights into microstructure, composition, and properties [145]. This versatile tool finds applications in polymer science and the investigation of nanomaterials like nanoparticles and 2D materials, offering valuable information about chemical composition, molecular orientation, and morphological characteristics. As a result, our understanding of the relationship between structure and properties has markedly improved [146], driving the development of innovative materials with customized properties for diverse applications.

Infrared microscopy enables high-resolution imaging and spectroscopic analysis at the nanoscale, facilitating a deeper understanding of size, shape dynamics, and surface properties [46]. It is particularly valuable for analyzing thin films or coatings used in solar cells, sensors, and protective coverings, providing detailed information on parameters such as thickness, chemical composition, and interfacial interactions [147]. This critical data empowers improvements in these elements, leading to enhanced efficiencies across various fields.

This transformative imaging technique has made significant strides in materials science by offering non-destructive evaluation of flaws such as cracks, voids, and inclusions in materials. Additionally, it provides valuable insights into temperature-dependent phenomena like phase transitions and thermal properties [110], enabling the development of materials with specific thermal characteristics [148].

Table 5. A comprehensive review of the use of FTIR, Raman, and Near-field Infrared Microscopy techniques in biomedical research. It details applications across various sample types and research areas, such as neurodegenerative diseases and pathogen identification, discussing the advantages and limitations of each technique within specific contexts, supported by relevant citations. The aim is to provide a comparative perspective on the utility of these microscopy methods in biomedicine.

Application	Technique	Sample Type	Advantages	Limitations	Reference
Neurodegenerative disease characterization	FTIR, Raman	Brain tissue sections	Detection of protein misfolding; Identification of biochemical markers	limited spatial resolution; Sample variability	[132]
Drug delivery and tracking	FTIR, Raman	Cells, tissues	Monitoring drug distribution; Evaluating drug efficacy and safety	Limited penetration depth; Sensitivity to high molecular weight compounds	[133]
Stem cell differentiation	FTIR, Raman	Stem cells	Non-destructive; Label-free; Real-time monitoring; Quality control	Limited sensitivity for rare cell types; Interpretation of complex spectra	[134]
Imaging of lipid distribution	FTIR, Raman	Cells, tissues	High chemical specificity; High spatial resolution	Limited penetration depth; Sensitivity to sample thickness	[135, 136]
Monitoring cell apoptosis	FTIR, Raman	Cells	Early detection of apoptosis; Label-free; High specificity	Limited sensitivity for early-stage apoptosis; Interpretation of complex spectra	[137]
Investigation of protein structure	FTIR, Raman	Protein samples	Determination of secondary structure; Evaluation of folding/unfolding	Limited sensitivity for low-abundance proteins; Challenges in data interpretation	[138]
Analysis of extracellular matrix composition	FTIR, Raman	Tissue sections	Detection of matrix components; Analysis of spatial distribution	Challenges in data interpretation; Limited spatial resolution	[139]
Pathogen identification	FTIR, Raman	Bacteria, fungi, viruses	Rapid identification; Label-free; Minimal sample preparation	Differentiation of closely related species; Limited spectral libraries	[140]
Atherosclerosis assessment	FTIR	Arterial tissue	Evaluation of plaque composition; Identification of vulnerable plaque	Invasive sample collection; Data processing and analysis	[141]
Metabolomics	FTIR, Raman	Cells, tissues, biofluids	Detection of metabolite signatures; Metabolic pathway analysis	Overlapping spectral features; Standardization of data processing	[142]
Cancer diagnosis and grading	FTIR, Raman, Near-field IR microscopy	Tissue sections	Non-destructive; Label-free; Molecular information for diagnosis; High sensitivity and specificity	Sample preparation; Data interpretation; Standardization	[143]
Imaging of subcellular organelles	Near-field IR microscopy	Cells	High spatial resolution; Label-free; Nanoscale imaging	Complex techniques; Limited penetration depth	[144]

Environmental Monitoring: Analyzing Microplastics and Atmospheric Particles

Infrared microscopy is revolutionizing environmental monitoring by providing valuable insights into the composition, distribution, and potential impacts of microplastics and atmospheric particles [148]. This section explores the diverse applications of infrared microscopy in enhancing our understanding of environmental concerns and facilitating efficient remediation strategies.

Microplastics, known for their environmental harm, are increasingly detectable through this advanced technique. Infrared microscopy offers crucial information about the size, shape, and chemical composition of particles. This data is essential for assessing associated risks and developing effective pollution reduction strategies [149].

In the realm of atmospheric particles, infrared microscopy plays a vital role. Researchers can glean valuable insights into their chemical structure, morphology, and size distributions. This information is critical for devising strategies to manage air quality and mitigate climate change impacts.

In bioaccumulation studies, infrared microscopy facilitates the investigation of microplastic accumulation and ecotoxicological implications [150]. Such insights are indispensable for safeguarding ecosystems from further damage.

Moreover, infrared microscopy proves highly efficient in detecting trace levels of contaminants such as persistent organic pollutants and heavy metals [151]. As such, it has become the preferred method for monitoring environmental conditions with its unique sensitivity and specificity, contributing significantly to environmental protection efforts.

Advanced Infrared Microscopy Techniques in the Analysis and Preservation of Art and Cultural Heritage

Infrared microscopy has significantly enhanced the examination of art and cultural heritage, providing scholars and conservators with a powerful tool to uncover intricate details of artworks and illuminate their artistic evolution over time [152]. This section delves into the various applications of infrared microscopy in art conservation, emphasizing its role in offering valuable insights into masterpieces and supporting their long-term preservation.

Reflectography stands out as a highly valuable non-invasive imaging technique used by experts to reveal hidden layers beneath the surface of artworks [153]. It enables the detection of underdrawings, pentimenti, and other subtle details that offer profound insights into an artist's creative process. These revelations serve as invaluable resources for art historians, aiding in authentication and enriching understanding of artistic intentions.

Furthermore, infrared microscopy enables the analysis of the chemical composition and distribution of pigments used in historical artworks [154]. Its capability to detect early signs of deterioration, such as cracks, delamination, and discoloration, is crucial for proactive conservation efforts [154, 155]. This technology plays a pivotal role in preserving cultural heritage by facilitating informed conservation decisions and ensuring the longevity of invaluable artworks.

Future Prospects and Ethical Considerations in Infrared Microscopy

Emerging Trends and Future Research Directions in Infrared Microscopy

The development of smaller and more portable designs has the potential to enhance the availability of advanced imaging capabilities for researchers in various contexts, including remote or resource-limited areas. This expansion could significantly broaden the application of such technology. However, there are concerns regarding the equitable access to these technologies. Additionally, integrating infrared microscopy with other advanced imaging techniques, such as Raman spectroscopy and X-ray fluorescence, enables comprehensive sample analysis, leading to valuable interdisciplinary advancements. Nevertheless, careful consideration is necessary for data management [156], interpretation, and distribution among interdisciplinary teams [157].

The integration of artificial intelligence (AI) and machine learning algorithms in infrared microscopy has the potential to revolutionize image analysis [27]. These technologies can accelerate discoveries and foster innovation by facilitating rapid and accurate identification of patterns and features within complex datasets. The rapid advancement of AI technology necessitates assessment of data privacy regulations, transparency standards, and potential biases in AI-driven analyses [158]. As infrared microscopy progresses swiftly, researchers and practitioners must address the ethical implications associated with its use, including obtaining informed consent and preventing misuse of information.

Achieving optimal utilization of the benefits offered by this powerful imaging technique, while effectively managing potential risks, requires collaborative efforts among all stakeholders, including the incorporation of AI-driven analyses [159].

Balancing Innovation and Privacy: Ethical Implications

Researchers must implement strict protocols for handling sensitive information, particularly when dealing with human tissue samples or proprietary substances. This is crucial to protect the rights and autonomy of individuals who consent to participate in research studies. Transparency is essential to establish trust and ethical standards. Providing study participants with a clear explanation of how infrared microscopy will be used enables them to make informed decisions about their participation in the investigation. Adhering to these guidelines prevents personal security breaches while engaging in significant scientific exploration using infrared microscopy.

Infrared microscopy has significantly advanced our understanding of the environment, providing unprecedented knowledge regarding the structure and dynamics of molecules. However, the vast potential of this technology also requires a higher level of accountability in safeguarding the privacy and security of individuals' data. To ensure ethical and responsible utilization of these advancements, researchers must establish precise research guidelines for infrared microscopy. Through open dialogue, we can collectively address the ethical implications of advances in infrared microscopy. This collaborative effort aims to promote responsible practices and optimize the potential for innovative discoveries. It facilitates a comprehensive analysis of the optimal balance between technological advancement and ethical concerns, such as genetic discrimination and unauthorized access to confidential data.

CONCLUSION

Infrared microscopy has emerged as a versatile and powerful technique with transformative potential across various scientific disciplines. This review provides a comprehensive examination of the methodologies, challenges, and emerging trends in infrared microscopy, offering a detailed analysis of the current landscape in this field. We have discussed key aspects such as sample preparation techniques and computational strategies for data interpretation, illustrating how recent advances are addressing existing challenges and enhancing the effectiveness and efficiency of this technology.

Furthermore, we have explored the diverse applications of infrared microscopy, highlighting its significant contributions to biomedical research, environmental monitoring, material science, and cultural heritage conservation. These advancements underscore its pivotal role in advancing scientific knowledge and facilitating innovative solutions in multiple domains.

As infrared microscopy continues to evolve with technological progress, ethical considerations surrounding data privacy and equitable access have become increasingly important. It is crucial for researchers to maintain ethical rigor and methodological robustness, particularly as the field integrates advancements like artificial intelligence. By leveraging infrared microscopy to its fullest potential, we can foster both scientific advancement and ethical responsibility.

This review serves as a testament to the achievements in the field of infrared microscopy and aims to guide future research efforts. It emphasizes the importance of a balanced approach that prioritizes both technological innovation and ethical considerations. Through collaborative efforts, the near future holds promise for groundbreaking discoveries and practical applications in infrared microscopy, shaping a remarkable era of scientific progress.

Conflicts of interest

The authors have stated that there is no conflict of interest associated with the publication.

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التحليل الطيفي المجهري بالأشعة تحت الحمراء: دراسة متعددة المجالات للتقنيات، الاستخدامات التطبيقية، والقضايا الأخلاقية

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ملخص

المجهرية الطيفية باستخدام الأشعة تحت الحمراء تعتبر واحدة من التقنيات الصاعدة التي لها تطبيقات واسعة في مجموعة متنوعة من الميادين العلمية. تستهدف هذه الدراسة متعددة التخصصات فحص متكامل للأساليب، التحديات، والابتكارات في هذا المجال. نقدم نظرة على أهمية طرق إعداد العينات، بما في ذلك التجزئة الكريوجينية والانعكاس الكلي المُضعف، في تحسين جودة البيانات. توجه الدراسة الأضواء على التحديات التي تواجهها عمليات الحصول على البيانات وتفسيرها، مع التركيز على حلول متقدمة مثل الخوارزميات المبنية على التعلم الآلي والمستشعرات الحساسة. الدراسة تكشف عن أوسع استخدامات المجهرية الطيفية في مجالات متعددة مثل الأبحاث الطبية، ومراقبة البيئة، وعلوم المواد، وحفظ الفنون. وتتعدى الدراسة الجوانب التقنية لتناقش التوجهات الجديدة، مثل دمج التكنولوجيا مع الذكاء الصناعي، والقضايا الأخلاقية كحماية البيانات والموافقة المُسبقة والمُستنيرة. تُقدم الدراسة مرجعاً شاملًا للباحثين، سواء كانوا متمرسين أو مبتدئين، عبر تقديم رؤية شاملة تجمع بين التطورات الأسلوبية والمعايير الأخلاقية.

الكلمات الدالة: التقنيات المجهرية الطيفية بالأشعة تحت الحمراء، إعداد العينات، تطبيقات متعددة المجالات، القضايا الأخلاقية، الخوارزميات المبنية على التعلم الآلي والذكاء الصناعي.

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