applied sciences

MDPI

Review

Artificial Intelligence and Machine Learning in AFM-Based Nanomechanical Biomarkers: From Force Curves and Stiffness Maps to Disease Classification and Treatment Monitoring

Andreas Stylianou

Cancer Mechanobiology and Applied Biophysics Group, School of Sciences, European University Cyprus, 2404 Nicosia, Cyprus; an.stylianou@euc.ac.cy

Abstract

Atomic force microscopy (AFM) has emerged as a powerful platform for quantifying nanoscale mechanical properties of cells, tissues, and extracellular matrix (ECM) components, providing candidate biomarkers for disease diagnosis, classification, prognosis, and treatment monitoring. However, the clinical translation of AFM-based nanomechanical biomarkers remains limited by low throughput, operator dependence, complex force-curve interpretation, heterogeneous biological samples, and the lack of standardized analytical pipelines. Artificial intelligence (AI) and machine learning (ML) approaches are increasingly being used to address these limitations by enabling automated AFM image and force-curve analysis, multiparametric feature extraction, cell and tissue classification, quality control, and high-throughput mechanophenotyping. This review summarizes how AI and ML have already been applied to AFM-derived nanomechanical and morphological data, with emphasis on cancer, fibrotic disease, and treatment response monitoring. We discuss classical ML models, deep learning approaches, clustering, fuzzy logic methods, and emerging automated Bio-AFM workflows. We further highlight current limitations, including small datasets, limited external validation, lack of reproducible reporting standards, and insufficient integration with clinical metadata. Finally, we propose a roadmap for AI-enabled AFM mechanobiomarkers, focusing on standardized datasets, explainable models, multimodal mechano-optical imaging, and clinically relevant validation strategies.

Keywords: atomic force microscopy (AFM); mechanobiology; deep learning; Young's modulus; mechanical heterogeneity; disease classification; treatment monitoring; mechanomics; precision medicine

Check for updates

Academic Editor: Thomas Lindner

Received: 17 June 2026
Revised: 22 July 2026
Accepted: 4 August 2026
Published: 5 August 2026

Copyright: © 2026 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

The mechanical phenotype of cells and tissues is now recognized as a fundamental regulator and reporter of biological function [1,2]. During physiological development, tissue repair, immune responses, fibrosis, and cancer progression, cells continuously sense and respond to mechanical cues generated by the extracellular matrix (ECM), neighbouring cells, and the surrounding microenvironment [3]. These cues are converted into biochemical signals through mechanotransduction pathways that regulate cytoskeletal organization, nuclear architecture, gene expression, proliferation, migration, invasion, and therapeutic response [2,4,5]. In pathological conditions, this mechanical homeostasis is frequently disrupted [6]. Cancer cells, stromal cells, fibrotic tissues, and remodeled ECM often acquire distinct mechanical and structural properties, suggesting that mechanical features can

provide complementary information to conventional molecular, histological, and imaging biomarkers [7–9].

For clarity, the terminology used in this review is defined early in the manuscript. "AFM measurements" refer to the primary experimental outputs generated by atomic force microscopy, including force–distance curves, force–indentation curves, stiffness maps, adhesion maps, viscoelastic maps, and topographical images. "AFM-derived descriptors" refer to quantitative parameters extracted from these measurements, such as Young's modulus, adhesion force, work of adhesion, roughness, viscoelastic parameters, force-curve features, and stiffness-distribution metrics. In this review, the terms "AFM-derived biomarkers" and "AFM-based nanomechanical biomarkers" refer to reproducible AFM-derived descriptors, or combinations of descriptors, that are associated with a biological or pathological state and may support disease classification, staging, prognosis, or treatment monitoring. When several AFM-derived descriptors are combined into a disease-associated mechanical profile, the terms "nanomechanical signature" or "nanomechanical fingerprint" are used. Unless explicitly stated otherwise, these biomarkers are considered candidate or emerging biomarkers rather than clinically validated biomarkers. Clinically validated biomarkers require analytical validation, biological reproducibility, independent cohort testing, and evidence of added value in clinically relevant workflows.

Atomic force microscopy (AFM) has played a central role in this emerging field because it enables nanoscale imaging and quantitative mechanical characterization of biological materials under near-physiological conditions [10]. In force spectroscopy and nanoindentation modes, AFM can measure the interaction between a sharp or colloidal probe and the sample surface, producing force–distance or force–indentation curves from which mechanical properties can be extracted using appropriate contact mechanics models [11]. Such measurements have been used to estimate Young's modulus, adhesion, work of adhesion, membrane tension, deformation, viscoelastic behavior, and spatial mechanical heterogeneity in single cells, cell clusters, ECM components, engineered biomaterials, and fresh or frozen tissue biopsies [7,12,13]. Unlike bulk mechanical testing, AFM provides local mechanical information at length scales relevant to the cytoskeleton, cell membrane, cell–matrix adhesions, collagen fibers, and heterogeneous tissue microdomains [14,15]. Therefore, AFM is uniquely positioned to bridge the gap between molecular-scale biophysics and tissue-level pathology.

The concept of AFM-based nanomechanical biomarkers has been developed most extensively in cancer research, where malignant transformation, metastatic potential, cytoskeletal remodeling, epithelial-to-mesenchymal transition, and drug response have been linked to measurable alterations in cell deformability, adhesion, and tissue mechanics [16]. At the tissue level, AFM has revealed that solid tumors often exhibit complex nanomechanical fingerprints, reflecting the coexistence of softer cancer-cell-rich regions and stiffer ECM-rich stromal compartments. These features are commonly represented by characteristic elasticity distributions, including lower elasticity peaks associated with softer cellular areas and higher elasticity peaks or broader high-stiffness components associated with collagen-rich or structurally remodeled tissue regions [7,8]. More recently, similar AFM-derived nanomechanical signatures have been proposed beyond cancer, including pulmonary fibrosis, where disease progression and antifibrotic treatment response are closely linked to ECM deposition, collagen remodeling, and tissue stiffening [9]. Together, these findings support the use of AFM-derived nanomechanical fingerprints as quantitative biomarkers for disease diagnosis, staging, prognosis, and treatment monitoring.

However, clinical translation remains challenging because AFM measurements are technically demanding, relatively slow, and sensitive to probe geometry, cantilever calibration, indentation depth, loading rate, sample preparation, hydration, temperature, and

data-processing assumptions. The extraction of Young's modulus and related parameters often requires fitting thousands of force curves to contact mechanics models, whose assumptions are not always valid for heterogeneous, adhesive, viscoelastic, poroelastic, anisotropic, and finite-thickness biological samples [7,17,18]. AFM datasets are intrinsically multidimensional, combining force curves, mechanical maps, images and statistical distributions, often alongside biological or clinical metadata. Reducing this information to simple descriptors such as mean or median stiffness may overlook disease-relevant features such as mechanical heterogeneity, spatial organization, distribution shape, multiparametric coupling, and subtle adhesive or topographical signatures. Therefore, advanced computational approaches are required to extract hidden patterns from complex AFM datasets and to support the development of reproducible, clinically meaningful nanomechanical biomarkers.

Artificial intelligence (AI) and machine learning (ML) are increasingly being explored as tools to address these limitations [17,19]. ML methods can identify complex relationships in high-dimensional datasets without requiring all features to be explicitly predefined by the investigator [20]. In AFM, ML has been used for force-curve analysis, image recognition, biomarker classification and acquisition automation [18,19,21]. AI/ML can support the full AFM workflow by guiding sample or region selection and probe positioning before acquisition, improving automation and throughput during measurement, and accelerating post-processing through reduced operator dependence and objective classification of AFM-derived nanomechanical signatures [17,19,22,23].

Several recent studies demonstrate the feasibility of AI-enabled AFM for biomedical classification and biomarker discovery. ML classifiers trained on AFM-derived mechanical properties have distinguished graded bladder cancer cells, showing that multiparametric mechanical phenotypes can outperform single descriptors [12]. Similarly, ML analysis of high-resolution AFM adhesion maps improved the discrimination between precancerous and cancerous cervical epithelial cells [24], neural-network analysis of AFM force–distance curves enabled automated cancer tissue diagnosis without laborious curve-by-curve fitting [25], and ML-based AFM image analysis differentiated cancer cells with different neoplastic aggressiveness [26]. Deep learning regressors trained on synthetic force–indentation curves have further shown that Young's modulus and adhesion energy can be predicted from AFM nanoindentation data, suggesting that physics-informed or synthetic-data-assisted learning may reduce the need for large experimental datasets [13]. This is particularly relevant because AFM datasets are often small, making approaches such as random forests, support vector machines, Gaussian process classifiers, shallow neural networks, dimensionality reduction, feature engineering, cross-validation, permutation testing, and robustness analysis especially important for biomedical AFM applications [21,27].

Beyond post-acquisition analysis, AI is increasingly being incorporated into AFM instrumentation and automation, including sample selection, scanning-site identification, probe navigation, image-quality assessment, autonomous acquisition, and high-throughput single-cell nanomechanical measurements [22,23,28]. Thus, AI-enabled AFM should not be viewed only as a tool for faster data processing, but as a route toward objective, automated, and multiparametric nanomechanical biomarker discovery. By integrating mechanical, adhesive, topographical, and spatial features, ML models can reveal disease- and treatment-associated patterns that may not be evident from individual parameters alone, supporting classification of disease stage, identification of aggressive phenotypes, prediction of therapeutic response, monitoring of tumor microenvironment normalization, fibrosis staging, antifibrotic treatment assessment, and quality control of engineered tissues or biomaterials.

However, substantial challenges remain before AI-assisted AFM nanomechanical biomarkers can be translated into clinically useful tools. Reliable translation will require standardized acquisition, calibration, analysis and reporting across laboratories, instru-

ments and data-processing pipelines [29,30]. In parallel, AI/ML models must be developed using rigorous validation strategies, including independent test datasets, appropriate cross-validation, statistical significance testing, and careful control of data leakage, batch effects, and overfitting, which are especially important when datasets are small or acquired from limited experimental batches [31,32]. Explainability will also be essential when model outputs are intended to support diagnosis, prognosis, or treatment decisions [33], since clinically useful models should not only classify samples but also identify the AFM-derived mechanical, adhesive, topographical, or spatial features driving classification and relate them to interpretable biological mechanisms. Future studies should determine whether AFM-derived mechanical signatures add value when integrated with histopathology, molecular profiling, imaging and clinical outcome data [34]. Addressing these methodological, computational, and translational barriers will be critical for moving AI-assisted AFM from proof-of-concept studies toward reproducible and clinically meaningful nanomechanical biomarker platforms.

The novelty of this review lies in its focused integration of AFM-derived nanomechanical biomarker discovery with AI/ML-assisted data analysis, rather than reviewing AFM mechanobiology or biomedical AI as separate topics. In contrast to broader reviews, this article critically connects AFM data types, computational methods, disease applications, validation requirements, explainability, standardization, and clinical-translation barriers into a single roadmap for AI-enabled AFM mechanobiomarker development.

In this review, we discuss how AI and ML have already been used in the field of AFM-based nanomechanical biomarkers, with emphasis on disease classification, cancer diagnosis, single-cell mechanophenotyping, tissue-level nanomechanical fingerprints, force-curve analysis, AFM image recognition, treatment monitoring, and automated AFM workflows. We first introduce the major AFM data types that can be used for ML analysis, including force curves, elasticity maps, adhesion maps, topographical images, and multiparametric mechanical datasets. We then summarize classical ML, deep learning, and physics-informed approaches that have been applied to AFM data. Subsequently, we discuss disease-oriented applications, focusing primarily on cancer and fibrotic diseases, where AFM-based mechanical biomarkers have shown particular promise. Finally, we highlight current limitations and propose future directions toward standardized, explainable, reproducible, and clinically relevant AI-enabled AFM mechanobiomarkers.

2. Methodology

This article was prepared as a narrative review. The aim was to provide a broad and critical overview of the emerging use of artificial intelligence (AI) and machine learning (ML) in atomic force microscopy (AFM)-based nanomechanical biomarker research, with emphasis on biomedical and clinically relevant applications.

The literature search was performed using combinations of keywords related to AFM, nanomechanics, AI/ML, and biomedical applications. Search terms included “atomic force microscopy”, “AFM”, “nanomechanical biomarkers”, “nanomechanical fingerprints”, “artificial intelligence”, “AI”, “machine learning”, “ML”, “deep learning”, “neural networks”, “support vector machine”, “random forest”, “clustering”, “fuzzy logic”, “force curve analysis” and “disease classification”. Searches were performed using major scientific databases and search engines, including PubMed, Scopus and Google Scholar. In addition, reference lists of relevant articles were manually screened to identify further studies not captured by the initial keyword searches.

No strict time restriction was applied, in order to include both foundational AFM mechanobiology studies and more recent AI/ML-based developments. However, priority was given to recent publications when discussing emerging computational methods, au-

tomation, high-throughput AFM, explainable AI, validation, and translational perspectives. Only articles published in English were considered. Included studies were selected based on their relevance to biological or medical applications of AFM, especially studies involving cells, tissues, extracellular matrix, disease classification, nanomechanical biomarkers, treatment response, or AI/ML-assisted AFM data analysis.

Articles were excluded if they were not related to AFM or nanomechanical analysis, focused exclusively on non-biological materials without biomedical relevance, did not include relevant AFM-derived mechanical, morphological, imaging, or force-curve data, or discussed AI/ML methods without a clear connection to AFM-based biological or medical applications. Conference abstracts, editorials, non-peer-reviewed sources, and studies with insufficient methodological or biological detail were generally not prioritized unless they provided important contextual information.

3. AFM-Derived Nanomechanical Biomarkers

AFM provides a unique experimental platform for the quantitative assessment of nanoscale and microscale mechanical properties of biological samples [7,10,35]. In contrast to bulk rheology, elastography, or macroscopic indentation techniques, AFM can probe local mechanical properties at length scales relevant to cell membranes, cytoskeletal structures, cell–matrix adhesions, extracellular matrix (ECM) fibers, and heterogeneous tissue microdomains [35,36]. Depending on the operational mode and the type of probe employed, AFM can generate force–distance curves, force–indentation curves, elasticity maps, adhesion maps, topographical images, deformation maps, viscoelastic parameters, and multiparametric nanomechanical datasets [37,38]. These outputs can define candidate nanomechanical biomarkers across healthy, diseased, and engineered biological systems [7,17,18,39,40].

In force spectroscopy or nanoindentation mode, the AFM probe approaches the surface of a compliant sample, indents it to a defined depth or force threshold, and then retracts. During this process, cantilever deflection is recorded as a function of the vertical displacement of the piezoelectric scanner. The cantilever deflection is converted into force through Hooke's law, while indentation is calculated by accounting for the movement of the probe and the deformation of the cantilever. These curves encode elastic, adhesive, dissipative, and time-dependent mechanical information. The approach part of the curve is most commonly used to estimate elastic properties, whereas the retraction part can provide information on adhesion, energy dissipation, and interaction forces between the probe and the sample. When thousands of force curves are acquired across a defined area, spatially resolved maps of mechanical properties can be generated [7,13].

3.1. Young's Modulus as the Most Widely Used AFM Nanomechanical Biomarker

Among all AFM-derived parameters, Young's modulus remains the most frequently used descriptor of biological sample mechanics [41]. Young's modulus describes resistance to deformation and is commonly used as an apparent stiffness parameter. In AFM experiments, it is usually extracted by fitting the approach segment of force–indentation curves to contact mechanics models. The Hertz model and its Sneddon modifications are the most widely applied frameworks, with model selection depending largely on probe geometry, indentation depth, sample properties, and the presence or absence of adhesive interactions [7,17,42–44].

Young's modulus has been used extensively as a biomarker of cancer-related mechanical alterations. At the single-cell level, malignant transformation is often associated with cytoskeletal remodeling and increased deformability, resulting in lower Young's modulus values compared with normal or less aggressive cells [45,46]. This cancer-associated softening

may facilitate migration, invasion, and metastatic dissemination [7]. However, the relationship between malignancy and cell stiffness is not universal [47]. Depending on cancer type, cell state, substrate stiffness, measurement region, indentation depth, and experimental conditions, malignant cells may exhibit different stiffness trends [48–50]. Therefore, Young's modulus alone should not be considered a universal stand-alone biomarker for all cancer types or biological systems [7,12].

At the tissue level, Young's modulus can provide complementary information to single-cell measurements. Many solid tumors stiffen during progression as a result of ECM remodeling, collagen deposition, fiber crosslinking, stromal activation, and increased structural heterogeneity [8,51]. Consequently, AFM studies of tumor biopsies often reveal complex elasticity distributions rather than a single representative stiffness value. In such tissues, lower elasticity values may correspond mainly to cell-rich or softer regions, whereas higher elasticity values may reflect collagen-rich, fibrotic, or stromal compartments [52,53]. This coexistence of cellular softening and matrix stiffening supports the use of full elasticity distributions rather than only mean or median Young's modulus values [7,8,52,53].

Young's modulus has also been proposed as a nanomechanical biomarker in fibrotic diseases, where progressive ECM deposition, collagen remodeling, and tissue stiffening can generate measurable shifts in local elastic properties [54–56]. Recent AFM studies in pulmonary fibrosis demonstrated that tissue nanomechanical signatures can characterize disease stage and monitor response to antifibrotic treatment. In this context, Young's modulus distributions can reflect progressive ECM remodeling and may provide information that complements histopathology, collagen imaging, and molecular readouts [9].

Despite its widespread use, Young's modulus is an apparent mechanical parameter that depends on experimental and analytical assumptions. Biological samples deviate from ideal elastic half-spaces because they are heterogeneous, adhesive, viscoelastic, poroelastic, anisotropic, and finite in thickness [57]. As a result, Young's modulus values can vary with indentation depth, probe geometry, loading rate, contact-point determination, model selection, sample preparation, and data-processing pipeline [41,42]. For this reason, Young's modulus should be interpreted as an operational biomarker that is meaningful when measured under standardized and reproducible conditions, rather than as an absolute material constant independent of context [7,18].

3.2. Adhesion and Work of Adhesion

Adhesion represents another important AFM-derived biomarker, particularly for probing cell-surface properties, cell–matrix interactions, extracellular vesicles, and soft biological materials, where nanoscale adhesive forces can reflect changes in molecular composition, receptor–ligand interactions, membrane organization, and pathological state [58,59]. During the retraction phase of an AFM force curve, adhesive interactions between the probe and the sample can generate negative force peaks before complete detachment. These events can be quantified using parameters such as maximum adhesion force, work of adhesion, adhesion energy, number of rupture events, detachment distance, and energy dissipation. Adhesion measurements are therefore closely linked to membrane composition, glycocalyx organization, receptor–ligand interactions, ECM binding, migration, and metastatic behavior [13,17].

In cancer mechanobiology, altered cell adhesion is closely linked to invasive behavior, epithelial-to-mesenchymal transition (EMT), metastatic potential, and therapeutic response, reflecting the central role of adhesion-mediated signaling in tumor-cell plasticity and interaction with the surrounding microenvironment [60,61]. AFM-based adhesion maps and force spectroscopy measurements can capture alterations in surface adhesiveness that may not be visible in conventional microscopy. For example, high-resolution AFM adhesion

maps have been used to distinguish precancerous from cancerous cervical epithelial cells when combined with machine learning analysis, demonstrating that adhesion-derived surface patterns may carry clinically relevant phenotypic information [24]. Similarly, multiparametric AFM studies have shown that adhesion and work of adhesion can contribute to classification models when combined with stiffness, membrane tension, and other cellular mechanical properties [12].

Adhesion is also important from an analytical perspective because the presence of adhesive forces influences the choice of contact model. Although Hertzian contact models are widely used to estimate elasticity from the approach segment of AFM force–indentation curves, they neglect adhesive interactions and may therefore be insufficient when probe–sample adhesion is significant. In such cases, adhesion-aware contact models, including the Johnson–Kendall–Roberts (JKR) and Derjaguin–Muller–Toporov (DMT) models, can provide more appropriate descriptions of the probe–sample interaction, with model selection depending on factors such as adhesion strength, sample compliance, tip radius, and interaction range [43,62,63]. In soft biological samples, where adhesion can be strong and short-ranged, the use of adhesion-aware approaches can improve the interpretation of retraction curves and enable the extraction of additional biomarker-relevant parameters [13,14,17].

3.3. Viscoelasticity and Time-Dependent Mechanical Behavior

Biological materials are not purely elastic. Cells and tissues exhibit time-dependent mechanical behavior because of cytoskeletal remodeling, intracellular fluid flow, poroelasticity, ECM relaxation, membrane viscosity, and dynamic molecular interactions. AFM can probe these properties through stress relaxation, creep, oscillatory indentation, multifrequency modes, force modulation, and viscoelastic mapping [64,65]. In such measurements, the response of the sample depends not only on the magnitude of the applied force or indentation but also on the rate and duration of loading [7,17].

Viscoelastic parameters may provide biomarker information that is not captured by Young's modulus alone. For example, two samples may have similar apparent stiffness under a specific indentation condition but differ in their relaxation time, energy dissipation, loss modulus, or rate-dependent response [66]. These differences can reflect cytoskeletal architecture, cell contractility, ECM organization, hydration, crosslinking, or tissue degradation. In cancer, fibrosis, and other mechanically active diseases, time-dependent mechanical behavior may be particularly relevant because pathological remodeling often affects both the elastic and dissipative components of the tissue [67]. Thus, viscoelasticity can enrich AFM-derived biomarkers by describing deformation, relaxation, energy dissipation, and recovery over time.

For AI/ML analysis, viscoelastic descriptors add biologically relevant dimensions beyond apparent stiffness. Instead of using a single scalar stiffness value, ML models can incorporate relaxation curves, loading-rate-dependent stiffness, hysteresis, dissipated energy, and frequency-dependent mechanical parameters. These descriptors may improve classification of cell states or tissue phenotypes, particularly in cases where elastic modulus alone is insufficient. However, viscoelastic AFM measurements also require careful standardization because they are sensitive to acquisition frequency, indentation depth, dwell time, ramp speed, probe geometry, and environmental conditions.

3.4. Surface Roughness and Nanotopographical Descriptors

AFM is not limited to mechanical measurements; it also provides high-resolution topographical imaging of biological surfaces. Surface height maps can provide quantitative descriptors of roughness, cell morphology, membrane protrusions and ECM ultra-

structure [68,69]. These nanotopographical descriptors are relevant because pathological transformation often alters surface organization, membrane architecture, collagen structure, ECM packing, and cellular morphology [7,13].

In cancer, AFM nanotopography has been used to investigate changes in cell surface morphology, membrane roughness, and tissue ultrastructure. Increased or decreased roughness may reflect cytoskeletal remodeling, apoptosis, altered glycocalyx, membrane blebbing, ECM degradation, or collagen reorganization. In fibrotic tissues, topographical features such as collagen fiber organization, D-banding, fiber packing, and surface irregularity may provide information on ECM remodeling and disease progression. Together with mechanical maps, these descriptors contribute to multiparametric AFM fingerprints.

Nanotopographical data are particularly compatible with machine learning analysis because AFM images are inherently digital, quantitative, and spatially resolved. Unlike many optical images, several AFM channels represent absolute or semi-quantitative physical properties, including height, adhesion, deformation, and stiffness. This makes them suitable for direct use in image classification, feature extraction, texture analysis, and multidimensional phenotyping [21]. However, AFM topographical images are also affected by artifacts such as tip convolution, sample roughness, drift, feedback parameters, and probe contamination. Therefore, roughness and other surface descriptors should be interpreted in relation to acquisition settings and probe condition.

3.5. Stiffness Distributions and Elasticity Spectra

In heterogeneous biological samples, the distribution of Young's modulus values is often more informative than a single average stiffness value. Cells, tissues, and ECM-rich specimens are spatially heterogeneous, and AFM force mapping can capture this heterogeneity by collecting hundreds or thousands of force curves over defined regions. The resulting elasticity spectra can reveal multiple mechanical subpopulations, such as softer cellular areas and stiffer ECM-rich regions. In tumor tissues, this has led to the concept of lower elasticity peaks and higher elasticity peaks or distributions, which can be associated with different tissue components and disease-related remodeling [7,8].

Disease progression may alter not only mean stiffness but also distribution shape, width, skewness, multimodality, and relative mechanical subpopulations. For example, tumor progression can broaden the stiffness distribution as cellular and stromal regions become more mechanically distinct. Treatment with microenvironment-normalizing agents or chemotherapeutics can shift these distributions, reduce high-stiffness components, or modify the balance between lower and higher elasticity regions. Similarly, in pulmonary fibrosis, progression and antifibrotic treatment response can be reflected by shifts in tissue stiffness distributions that correlate with collagen content and histological remodeling [8,9].

These distribution-level features are highly relevant for AI-enabled biomarker discovery. Machine learning models can use entire distributions or extracted statistical descriptors, including mean, median, variance, standard deviation, coefficient of variation, percentiles, peak position, peak amplitude, distribution width, skewness, kurtosis, and mixture-model parameters. Such features may capture mechanical heterogeneity more effectively than single-value summaries. In this sense, stiffness distributions represent an important transition from simple stiffness measurement to AFM-based mechanomics.

3.6. Force-Curve Descriptors Beyond Fitted Mechanical Parameters

Although many AFM studies focus on parameters derived from contact-model fitting, the raw force curve itself contains additional information that may be biologically meaningful. Force-curve descriptors can include contact-point position, slope, indentation depth, maximum force, hysteresis area, adhesion force, pull-off distance, number of rupture events,

baseline noise, curve shape, nonlinearity, relaxation behavior, and fitting residuals [13,70]. Some of these features may reflect sample mechanics directly, whereas others may indicate surface interactions, sample heterogeneity, probe contamination, or measurement quality.

The use of force-curve descriptors is becoming increasingly important in machine learning applications. Rather than extracting only Young's modulus or adhesion from each curve, ML algorithms can be trained directly on raw or preprocessed force–distance and force–indentation data. This approach can reduce reliance on potentially imperfect contact models and may reveal subtle shape-based features that conventional fitting ignores. Neural networks and other ML models have already been used to classify tissues or predict mechanical properties directly from force curves, suggesting that raw AFM curves may serve as information-rich inputs for automated biomarker discovery [25].

However, force-curve-based ML must be approached carefully. Raw curves are sensitive to baseline correction, contact-point detection, sampling rate, noise, indentation range, probe calibration, and preprocessing choices. If these factors are not standardized, ML models may learn instrument- or protocol-specific artifacts rather than biologically meaningful patterns. Therefore, force-curve descriptors should ideally be combined with rigorous quality control, transparent preprocessing, external validation, and explainable model interpretation.

Raw AFM force–distance or force–indentation curves can be used directly as inputs to ML models, rather than being reduced only to fitted Young's modulus values. In this approach, each curve is treated as an information-rich signal containing indentation slope, adhesion events, hysteresis, relaxation behaviour, baseline features, and curve-shape descriptors. Neural networks and deep learning regressors can learn diagnostic patterns from complete curves, which may reduce dependence on contact-model assumptions and manual curve fitting [70].

3.7. Mechanical Heterogeneity as a Biomarker

Mechanical heterogeneity is a defining feature of many biological systems and diseases. A tissue is not mechanically uniform; it contains cells, ECM fibers, blood vessels, stromal compartments, necrotic regions, inflammatory areas, and mechanically distinct microdomains. Similarly, a single cell contains regions with different cytoskeletal organization, nuclear stiffness, membrane tension, and adhesion properties. AFM can capture this heterogeneity because it measures mechanical properties locally and can generate spatial maps across cells or tissues [7,8].

In cancer and fibrosis, mechanical heterogeneity reflects softer cell-rich and stiffer collagen-rich regions, generating broad elasticity spectra linked to invasion, transport, mechanotransduction, therapy resistance, and treatment response. Therefore, mechanical heterogeneity itself can be considered a biomarker, not merely a source of experimental variability [8,9].

Quantifying heterogeneity requires approaches that preserve spatial and distributional information. Useful descriptors include local stiffness gradients, spatial autocorrelation, texture features of elasticity maps, clustering of mechanical regions, entropy, variance, distribution multimodality, and correlation between stiffness and structural features such as collagen content. These features are particularly suitable for machine learning analysis because they allow the model to incorporate spatial organization and multiparametric patterns. Thus, AI-enabled AFM analysis may help convert mechanical heterogeneity from a technical challenge into a biologically meaningful biomarker.

3.8. Toward Multiparametric AFM Mechanobiomarkers

The most informative AFM-derived biomarkers are unlikely to be based on a single parameter. Young's modulus, adhesion, viscoelasticity, roughness, stiffness distributions, force-curve descriptors, and mechanical heterogeneity describe different aspects of the biological sample (Figure 1). Each parameter captures only part of the underlying phenotype. For example, Young's modulus may reflect resistance to indentation, adhesion may reflect surface chemistry and receptor interactions, roughness may describe topographical remodeling, and viscoelasticity may reveal time-dependent dissipative behavior. Combining these parameters can generate a more comprehensive nanomechanical fingerprint.

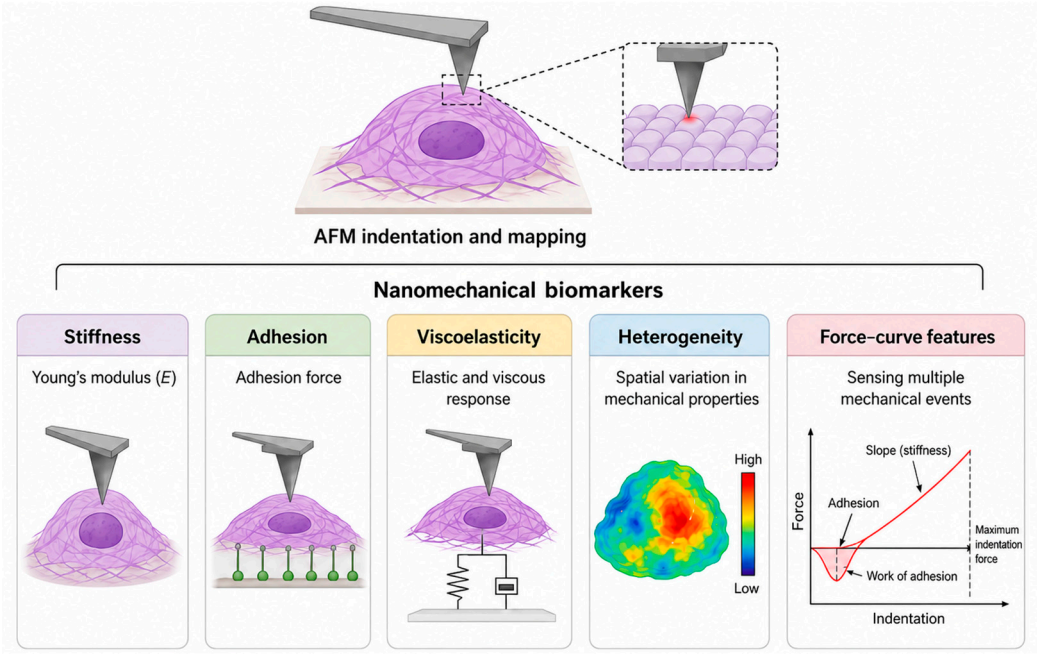


Figure 1. Overview of AFM-derived nanomechanical biomarkers. AFM indentation and mapping of biological samples generate key nanomechanical biomarkers, including Young's modulus, adhesion, viscoelasticity, and mechanical heterogeneity. Together, these multiparametric readouts define nanomechanical fingerprints that can be used for cell and tissue phenotyping, disease classification, and treatment monitoring. A multiparametric AI model should ideally include complementary AFM-derived features rather than a single mechanical descriptor. Relevant inputs may include Young's modulus, adhesion force, work of adhesion, viscoelastic descriptors, roughness, force-curve hysteresis, stiffness-distribution parameters, and spatial heterogeneity. The most appropriate feature set depends on the biological question: stiffness and adhesion may be most relevant for cell classification, distributional and spatial features for tissue heterogeneity, and viscoelastic or hysteresis descriptors for treatment response monitoring [8,12,24].

This multiparametric concept is particularly important for AI and ML applications. Machine learning models can integrate multiple AFM-derived features and identify patterns that are not obvious when parameters are analyzed separately. Such approaches may improve classification accuracy, reveal disease subtypes, predict treatment response, and support biomarker discovery. In this context, AFM-derived nanomechanical biomarkers should be viewed not as isolated measurements but as components of a broader mechanobiological signature that includes mechanical, adhesive, topographical, spatial, and temporal information [12,21,24].

The development of AFM-derived biomarkers will require standardized protocols, robust computational analysis, and biological validation. Together, these advances can

transform AFM from a specialized mechanical characterization technique into a quantitative platform for nanomechanical biomarker discovery.

4. AI/ML Methods Used in AFM Biomarker Studies

The increasing use of AI and ML in AFM reflects the need to extract biologically meaningful information from complex, high-dimensional datasets that include force–distance curves, elasticity and adhesion maps, topographical images, roughness descriptors, viscoelastic parameters, and spatial mechanical distributions [17,19,21]. While conventional analysis based on manual preprocessing, curve fitting, user-defined thresholds, and selected parameters such as mean or median Young's modulus has provided important mechanobiological insights, it may overlook subtle, nonlinear, and multiparametric patterns associated with disease states, cell phenotypes, or treatment responses [12,24]. Classical ML algorithms can classify samples using engineered AFM features such as stiffness, adhesion, roughness, texture and force-curve descriptors [13,19,24].

4.1. Classical Supervised Machine Learning Methods

Classical supervised ML methods are particularly relevant for AFM biomarker studies because AFM datasets are often small, reflecting slow acquisition, laborious sample preparation, limited patient material, and the need for expert handling [21]. Algorithms such as support vector machines, logistic regression, k-nearest neighbors, naïve Bayes classifiers, decision trees, random forests, Gaussian process classifiers, and shallow neural networks can therefore be useful for classification tasks based on AFM-derived features, including Young's modulus, adhesion, roughness, stiffness-distribution parameters, image texture features, and force-curve descriptors [26,71,72].

Support vector machines are well suited to small-to-medium AFM datasets because they can separate classes in high-dimensional feature spaces and have already been used in mechanobiological studies involving AFM-derived nanomechanical fingerprints, including pulmonary fibrosis staging with AFM and optical microscopy-derived parameters [9]. Linear classifiers such as logistic regression can provide more interpretable feature weights, helping identify whether classification is driven by stiffness, adhesion, roughness, or mechanical heterogeneity. Simpler models such as k-nearest neighbors and naïve Bayes can serve as useful baselines, although correlated AFM features may limit their biological interpretability. Gaussian process classifiers are also relevant because they provide probabilistic predictions and uncertainty estimates, and have been applied to AFM images to differentiate cancer cells with different neoplastic aggressiveness [26,69].

Supervised ML methods can be applied when AFM-derived measurements are linked to known biological labels, such as healthy versus diseased, low- versus high-grade cancer, fibrosis stage, or treated versus untreated samples. Algorithms such as SVM, logistic regression, random forest, and gradient boosting can use engineered AFM features to classify samples, rank informative biomarkers, and identify multiparametric mechanical signatures associated with disease progression or treatment response. Such models are particularly useful when stiffness, adhesion, force-curve descriptors, and distributional features provide complementary information [9,12,24].

4.2. Ensemble Learning

Ensemble learning methods, including random forests, gradient boosting, adaptive boosting, and related approaches, are useful in AFM biomarker studies because they can handle nonlinear relationships, mixed feature types, noisy measurements, and interactions among multiple AFM-derived descriptors [19,73]. They can also provide feature-importance estimates, helping identify which parameters contribute most strongly to classi-

fication. In AFM-based cervical-cell analysis, random forest classification of high-resolution adhesion maps improved the discrimination between precancerous and cancerous human epithelial cervical cells compared with earlier single-parameter fractal analysis, highlighting the value of ensemble learning for clinically relevant AFM image-based classification [24].

Ensemble methods are also valuable when several mechanical parameters are measured simultaneously. In graded bladder cancer cells, AFM nanoindentation was used to extract cellular elastic modulus, work of adhesion, adhesiveness, and membrane tension, and ML analysis of these multiparametric mechanical phenotypes achieved better classification than single or dual descriptors [12]. Gradient boosting and related methods may further integrate weak but complementary features, such as stiffness heterogeneity, adhesion map texture, roughness, and force-curve hysteresis, although careful cross-validation, independent testing, and permutation-based significance assessment remain essential to avoid overfitting in small AFM datasets.

4.3. Artificial Neural Networks and Deep Learning

Artificial neural networks and deep learning methods are increasingly being applied to AFM data because they can learn nonlinear relationships and hierarchical features directly from raw or minimally processed inputs. In AFM studies, they can be trained on force–distance curves, indentation curves, topographical images, adhesion maps, stiffness maps, or synthetic data, enabling classification, regression, denoising, image recognition, curve analysis, and prediction of mechanical properties.

One important application is the direct analysis of AFM force–distance curves, which can reduce reliance on time-consuming contact-model fitting and operator-dependent decisions. A fully automated neural-network approach has been used for cancer tissue diagnosis, showing that force-curve information can distinguish tumor from non-tumor tissues and support rapid, operator-independent analysis [25]. Deep learning regressors trained on synthetic AFM nanoindentation curves generated from contact mechanics theories have also been shown to predict Young's modulus and adhesion energy from soft-sample force–indentation data, suggesting that physics-informed or simulation-assisted learning may help overcome the limited availability of large standardized experimental AFM datasets [13].

Convolutional neural networks (CNNs) and related architectures also have potential for AFM image classification, segmentation, artifact recognition, and multiparametric map analysis [21,74], although their use is constrained by the small size of most AFM image datasets and artifacts such as tip convolution, drift, contamination, feedback settings, and sample deformation [19,21]. Deep learning may be especially useful when multiple AFM channels, such as height, adhesion, stiffness, deformation, and dissipation, are treated as complementary image layers. However, careful validation is essential to ensure that models learn disease-relevant biological patterns rather than batch effects, sample-preparation artifacts, imaging settings, or instrument-specific signatures.

Deep learning models can also be trained on AFM stiffness maps, adhesion maps, topographical images, or multilayer AFM image channels. In such workflows, each AFM channel can be treated as a complementary image layer, allowing CNNs or related architectures to identify spatial disease patterns, local heterogeneity, mechanically abnormal regions, or map-level texture features. This strategy is particularly promising for AFM image recognition and multiparametric map analysis, although it requires careful control of scan artifacts, batch effects, sample preparation, and limited dataset size [13,70].

4.4. Fuzzy Logic and Rule-Based Computational Classification

Fuzzy logic provides a useful approach for AFM data analysis when mechanical classes and tissue boundaries are not sharply defined. Biological tissues often contain mixed regions that cannot be classified strictly as cellular, stromal, fibrotic, necrotic, or ECM-rich. By allowing each data point to have partial membership in multiple classes, fuzzy clustering or rule-based analysis can better represent gradual mechanical transitions and heterogeneous tissue compartments [75,76].

This approach is particularly relevant for AFM tissue mapping, where stiffness measurements may arise from cancer cells, stromal cells, collagen fibers, blood vessels, adipose regions, or mixed microdomains. Earlier AFM studies of breast cancer tissue used fuzzy-logic-based clustering to categorize Young's modulus measurements into mechanically meaningful regions, supporting the use of computational classification to distinguish multiple tissue components rather than only normal and malignant regions [7]. Although interpretable and compatible with biological heterogeneity, fuzzy logic depends on the choice of membership functions, cluster number, and classification rules, and should therefore be validated against histology, immunostaining, collagen imaging, or molecular markers.

4.5. Unsupervised Learning and Clustering

Unsupervised learning is useful in AFM biomarker studies when class labels are unavailable, incomplete, or not the main focus of the analysis. For example, self-organizing maps have been applied to AFM-based viscoelastic characterization of breast cancer cell mechanics [77], while chemometric tools such as PCA and hierarchical clustering have been used to extract hidden structure from AFM force-spectroscopy datasets [78]. More broadly, k-means clustering, hierarchical clustering, Gaussian mixture models, self-organizing maps, PCA, t-distributed stochastic neighbor embedding, uniform manifold approximation and projection, and density-based clustering can reveal natural groupings, mechanical subpopulations, outliers, and disease-related patterns within complex AFM datasets.

Clustering is particularly relevant for stiffness distributions, elasticity maps, and AFM images because biological samples often contain mechanically distinct regions. In tumor tissues, clustering of AFM-derived mechanical data has been used to distinguish mechanically different breast tissue regions [75], while k-means and fuzzy C-means clustering have been applied to pathological ovarian tissues to separate mechanically distinct tissue components [76]. In fibrotic tissues, similar approaches could distinguish mechanically preserved areas from collagen-dense stiff regions, whereas at the single-cell level clustering may reveal mechanical phenotypes associated with differentiation state, metastatic potential, drug response, or cytoskeletal organization.

Gaussian mixture models are especially useful when AFM stiffness distributions are multimodal, allowing tissues to be represented as combinations of distinct mechanical populations rather than by a single mean modulus. In this context, AFM studies of cancer-affected breast tissue have shown that distribution-level mechanical analysis can reveal mechanically distinct tissue components [75], while pathological ovarian tissue studies further support the use of clustering-based analysis of AFM elasticity data [76]. The position, amplitude, width, and relative weight of these populations can serve as distribution-level biomarkers and may be used as inputs for supervised classification or treatment response prediction. However, unsupervised clusters still require biological validation through microscopy, histology, molecular markers, or experimental perturbations to avoid assigning meaning to technical artifacts.

Unsupervised learning can reveal hidden mechanical subpopulations in heterogeneous tumour or fibrotic tissues without requiring predefined labels. PCA can reduce correlated AFM variables and visualize sample separation, while k-means clustering, Gaus-

sian mixture models, t-SNE, and UMAP can help identify mechanically distinct regions or cell populations. These approaches are useful for multimodal stiffness distributions, where different clusters may correspond to softer cellular regions, stiffer ECM-rich compartments, or treatment-modified mechanical states [75,77,78].

4.6. Dimensionality Reduction and Feature Engineering

Dimensionality reduction is important in AFM-ML workflows because AFM datasets may contain many features, including raw pixels, image textures, force-curve descriptors, stiffness-distribution parameters, adhesion metrics, viscoelastic values, and topographical descriptors. When feature number is high relative to sample size, models become vulnerable to overfitting, making dimensionality-reduction and feature-extraction strategies important for AFM image and data analysis [79]. Methods such as PCA, manifold learning, and automated feature extraction can compress AFM data into more informative representations while preserving relevant structural or mechanical patterns.

PCA is commonly used to reduce correlated AFM features into a smaller set of orthogonal components and to visualize whether samples separate naturally in reduced feature space. However, because PCA is linear and unsupervised, it may not always preserve the most diagnostic variables. Nonlinear or deep-learning-based approaches may capture more complex relationships in AFM image or force-curve analysis, as shown by CNN-based event detection in AFM force measurements [80] and deep learning classification from AFM images [74], but these approaches should usually be treated as exploratory unless externally validated.

Feature engineering remains especially important for small AFM datasets because biologically interpretable descriptors may be more reliable than end-to-end deep learning. Relevant features include median Young's modulus, stiffness variance, adhesion map roughness, elasticity-map entropy, histogram peak position, force-curve hysteresis, pull-off force, relaxation time, fiber orientation, fractal dimension, and spatial autocorrelation. Automated AFM image feature extraction can support more reproducible descriptor generation [81], but feature selection must be performed inside the training folds to avoid data leakage, and selected variables should be linked to plausible mechanisms such as cytoskeletal remodeling, collagen deposition, matrix stiffening, adhesion changes, or altered viscoelasticity.

4.7. Explainable AI and Model Interpretability

Explainable AI is essential for AFM-based biomarker studies because high classification accuracy alone is insufficient for clinical translation. In biomedical AI, interpretability supports trust, reproducibility, biological understanding, and regulatory acceptance [82]. For AFM applications, explainability can help determine whether classification is driven by meaningful nanomechanical features, such as stiffness heterogeneity, adhesion, viscoelasticity, or roughness, rather than technical artifacts.

For classical ML models, interpretability can be achieved using feature importance, model coefficients, decision rules, permutation importance, partial dependence analysis, or SHAP values [83]. Random forests and gradient boosting can rank the contribution of stiffness, adhesion, roughness, or distribution descriptors, while logistic regression can indicate whether each feature increases or decreases the probability of a disease class. Model-agnostic approaches such as LIME can also support local interpretation of individual predictions [84].

For deep learning models, explainability is more challenging but critical for AFM image, map, and force-curve analysis. Saliency maps, Grad-CAM, layer-wise relevance propagation, SHAP values, and occlusion analysis can identify whether predictions depend

on biologically relevant regions or curve segments, such as adhesion peaks, indentation slopes, hysteresis, membrane protrusions, or stiffness gradients [85,86]. This is also important for quality control, since explanations may reveal that a model relies on scan-line noise, image borders, baseline offsets, or instrument-specific patterns rather than true disease-related mechanics.

Explainable AI can help determine which AFM-derived features, image regions, or force-curve segments drive model predictions. For engineered-feature models, feature importance, permutation importance, SHAP, or LIME can identify whether classification depends mainly on stiffness, adhesion, roughness, viscoelasticity, or mechanical heterogeneity. For CNN-based analysis of AFM maps or images, saliency maps and Grad-CAM can highlight spatial regions that contribute to classification, such as stiff ECM-rich areas, adhesive surface domains, or mechanically heterogeneous tissue compartments. These approaches can increase biological interpretability and help detect cases where models rely on artifacts rather than disease-relevant nanomechanical information [83–85].

4.8. Model Validation, Robustness, and Statistical Significance

Although most AI/ML studies in AFM-based nanomechanical biomarker research currently rely on classical ML, shallow neural networks, CNNs, clustering, and engineered features, newer AI approaches may become important for future AFM workflows. Transformer-based models are increasingly used in medical image analysis and could be useful for analysing spatial patterns in stiffness maps, adhesion maps, and multiparametric AFM images [87]. Self-supervised learning is also particularly relevant because AFM datasets are often small, expensive to annotate, and rich in unlabelled images or force curves. By pretraining models on large unlabelled AFM datasets, useful representations could be learned before fine-tuning on disease classification, treatment response prediction, or quality-control tasks [88]. In the longer term, foundation-model approaches may support transfer learning across instruments, laboratories, sample types, and imaging modalities, especially when AFM data are integrated with histology, fluorescence microscopy, Raman spectroscopy, or molecular profiles [89]. However, these approaches remain largely unexplored in AFM-based biomarker studies and will require standardized datasets, external validation, interpretability, and careful control of batch effects before they can be considered translationally reliable.

The reliability of AI/ML methods in AFM biomarker studies depends strongly on validation. AFM datasets are often small, heterogeneous, and vulnerable to batch effects; therefore, high classification accuracy alone is not enough, especially because small-sample cross-validation can produce unstable or overly optimistic estimates [90]. Models should be assessed using appropriate validation, independent testing where possible, and performance metrics that capture both discrimination and calibration. Particular care is needed when multiple measurements are obtained from the same biological sample. If force curves or images from the same cell, tissue, patient, or animal appear in both training and test sets, the model may learn sample-specific features and produce overly optimistic performance, a form of data leakage that has been emphasized in machine-learning-based scientific studies [91].

Cross-validation should be designed according to the biological question. For example, if the goal is patient-level diagnosis, data splitting should be performed at the patient level, not at the force-curve or image level, because improper image- or measurement-level splitting can inflate model performance and reduce clinical validity [92]. If the goal is to classify cell lines, splitting should consider independent biological replicates. If the goal is treatment response prediction, the test set should include samples not used during

feature selection or model optimization. These considerations are essential for avoiding data leakage and for producing realistic estimates of translational performance.

Permutation testing and random-label shuffling are valuable tools for assessing whether the observed classification performance is statistically meaningful. This is particularly important in small AFM datasets, where models can sometimes appear accurate by chance. Recent discussions of ML analysis in AFM emphasize the importance of robustness analysis and statistical significance testing, especially when working with small image datasets [21]. Such practices should become standard in AI-enabled AFM biomarker studies.

Validation should be performed at the correct biological level. When multiple force curves or images are collected from the same cell, tissue, animal, or patient, measurements from the same biological source should not be split across training and test sets. Instead, splitting should be performed at the cell, tissue, animal, donor, or patient level, depending on the intended application. Feature selection, preprocessing, scaling, dimensionality reduction, and model tuning should also be performed inside the training folds to avoid data leakage. These precautions are essential because small biomedical datasets can produce unstable cross-validation estimates and overly optimistic performance when data are not properly separated [90–92].

4.9. Toward Integrated AI-AFM Biomarker Pipelines

The most promising AI/ML approaches in AFM biomarker research will likely combine several methods into integrated pipelines. As summarized in Figure 2, a typical workflow may include automated AFM acquisition, quality control of force curves and images, extraction of mechanical and topographical features, dimensionality reduction, supervised or unsupervised learning, explainable feature attribution, and validation against histology, molecular markers, and clinical outcomes. Table 1 complements this workflow by summarizing representative AFM data types, AI/ML approaches, biomedical applications, translational contributions, and key limitations. Recent reviews on AI/ML-enabled AFM biomarker translation [17] and machine learning approaches for AFM instrumentation and data analytics [19] further support the need for integrated, standardized, and scalable pipelines.

Table 1. Representative AI/ML approaches used in AFM-based nanomechanical biomarker studies. The table summarizes the AFM input data, AI/ML approach, biomedical application, key contribution, main limitation, and reported sample, dataset, validation, or performance information where available.

AFM Input Data	AI/ML Approach	Application	Key Contribution	Main Limitation	Sample/Dataset/ Validation/Performance	Ref.
Cellular mechanical features: Young's modulus, adhesion, membrane tension	Supervised ML	Graded bladder cancer cells	Multiparametric features improved cancer-grade classification	Cell-line based; patient validation needed	Cell samples; 1600 mechanical-property sets; accuracy 91.25%; AUC 0.9798	[12]
High-resolution adhesion maps	Random forest	Precancerous vs. cancerous cervical cells	Adhesion map features improved discrimination	One cancer type and one AFM modality	172 cells; AUC 0.93; accuracy 83%; sensitivity 92%; specificity 78%	[24]
Force–distance curves	Artificial neural networks	Brain tumour tissue classification	Automated classification directly from force curves	Needs broader instrument and tissue validation	15 brain-tumour patients; AUC 0.83–0.96 for approach curves and 0.76–0.97 for retract curves	[25]
AFM images and surface maps	Classical ML and statistical validation	AFM image classification	Provided a framework for small AFM image datasets	Small datasets remain vulnerable to overfitting	Methodological article; no single classifier performance value reported	[21]

Table 1. *Cont.*

AFM Input Data	AI/ML Approach	Application	Key Contribution	Main Limitation	Sample/Dataset/ Validation/Performance	Ref.
Force–indentation curves	Deep learning regression using synthetic data	Prediction of Young's modulus and adhesion energy	Synthetic curves trained regressors for AFM property prediction	Synthetic curves may not capture full biological complexity	40,000 synthetic curves; MAPE 0.67% on synthetic data and 6.14% on experimental data	[13]
Tissue stiffness distributions and nanomechanical fingerprints	Statistical/ mechanobiomarker analysis	Solid tumour treatment monitoring	Detected treatment-induced mechanical remodelling	Requires standardized tissue sampling	Treatment-monitoring study; no AI/ML classifier performance reported	[8]
AFM nanomechanical fingerprints with optical microscopy	SVM and in silico classification	Pulmonary fibrosis staging and drug response	Supported staging and pirfenidone-response monitoring	Broader clinical validation needed	Tissue-based study; SVM accuracy 79.5–98.5%	[9]
AFM instrumentation and image acquisition	ML-assisted automation and quality control	General AFM/Bio-AFM workflows	Summarized ML-supported acquisition and automation	Often sample- or instrument-specific	Review article; no new classifier performance reported	[19]
AFM-based cancer biomarkers	AI/ML translational review	Cancer diagnosis and prognosis	Positioned AI/ML as a route to clinical translation	Translational review, not new validation study	Review article; no new classifier performance reported	[17]

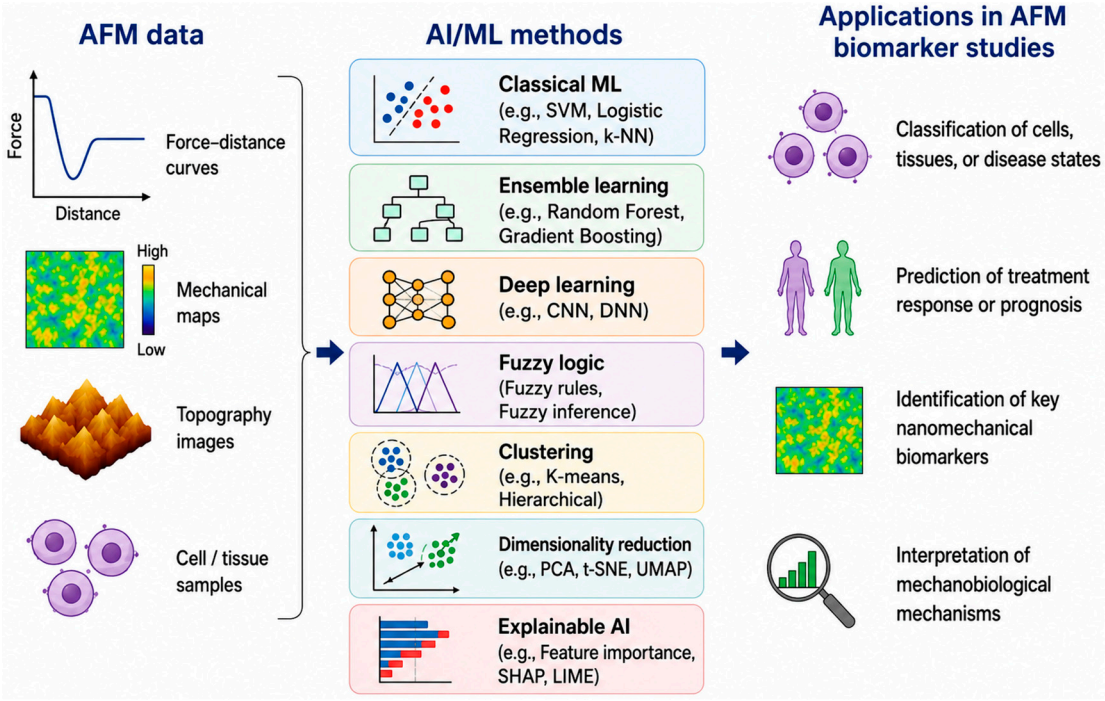


Figure 2. AI/ML methods used in AFM-based nanomechanical biomarker studies. AFM-derived datasets, including force–distance curves, mechanical maps, topographical images, and cell or tissue measurements, can be analyzed using different AI/ML approaches such as classical machine learning, ensemble learning, deep learning, fuzzy logic, clustering, dimensionality reduction, and explainable AI. These methods enable classification of cells, tissues, and disease states, prediction of treatment response or prognosis, identification of key nanomechanical biomarkers, and interpretation of underlying mechanobiological mechanisms. Overall, AI and ML provide a powerful computational framework for AFM-derived nanomechanical biomarker discovery. By integrating stiffness, adhesion, viscoelasticity, roughness, force-curve features, and mechanical heterogeneity, these methods can reveal complex mechanobiological signatures that are difficult to detect using conventional analysis,

including multiparametric cancer cell phenotypes [12] and adhesion-map-based discrimination of precancerous and cancerous cells [24]. However, successful application requires standardized acquisition, transparent preprocessing, careful validation, explainable interpretation, and biological confirmation so that AI-enabled AFM biomarkers can progress from computational classifiers to reliable tools for disease diagnosis, staging, prognosis, and treatment monitoring. AI-based quality control can be incorporated at multiple stages of the AFM workflow. Before analysis, ML models can flag poor force curves, baseline noise, unreliable contact-point detection, excessive drift, scan-line artifacts, probe contamination, or abnormal image features. During acquisition, automated models may also guide region selection, identify unsuitable measurements, and reduce operator dependence. Such quality-control steps are essential for high-throughput AFM mechanomics because unreliable curves or images can otherwise propagate into biased biomarker models [19,21,70].

Importantly, different AI/ML methods are suited to different tasks. Classical ML and ensemble learning are well suited to small-to-medium AFM datasets with engineered features, particularly when statistical significance and small-sample limitations are carefully considered [21]. Deep learning is promising for raw force curves, large image datasets, synthetic training data, and automated analysis, as shown by deep learning regressors trained on AFM nanoindentation curves [13]. In contrast, fuzzy logic and clustering are useful for heterogeneous tissue compartments, dimensionality reduction supports visualization and feature compression, and explainable AI is necessary for biological interpretation and clinical trust.

5. Materials and Methods Applications in Disease Classification

AFM-derived nanomechanical biomarkers can support disease classification because pathological transformation is often accompanied by local changes in stiffness, adhesion, viscoelasticity, topography, and mechanical heterogeneity arising from cytoskeletal remodeling, altered cell–matrix adhesion, ECM deposition, collagen crosslinking, stromal activation, inflammation, fibrosis, necrosis, or treatment-induced remodeling. When integrated with AI/ML methods, these multiparametric datasets can help distinguish normal and pathological samples, classify disease states or malignancy grade, identify mechanically distinct tissue subregions, and support diagnostic or prognostic assessment in cancer and other remodeling-driven diseases, including pulmonary and liver fibrosis, cardiovascular, neurodegenerative, and musculoskeletal disorders.

5.1. Classification of Cancer Cells Using AFM-Derived Mechanical Phenotypes

At the single-cell level, cancer development is associated with changes in cytoskeletal architecture, membrane tension, adhesion, nuclear mechanics, and cell deformability, which can be quantified by AFM nanoindentation and force spectroscopy. Although many cancer cells exhibit lower stiffness than non-malignant counterparts, cell stiffness alone is not a universal classifier because it depends on cell cycle, substrate stiffness, culture conditions, measurement region, indentation depth, and tumor-specific biology. This has motivated the use of multiparametric AFM phenotyping combined with ML, rather than reliance on individual mechanical descriptors.

A representative example is the classification of graded bladder cancer cells using AFM-derived cellular elastic modulus, work of adhesion, adhesiveness, and membrane tension. In this study, single or dual mechanical properties were insufficient to classify all cancer grades, whereas ML analysis of multiparametric mechanical phenotypes improved discrimination of cancerization stages and provided a more objective grading strategy [12]. Similarly, ML analysis of high-resolution AFM adhesion maps from human cervical epithelial cells improved the distinction between precancerous and cancerous phenotypes compared with earlier single-parameter fractal analysis, demonstrating that adhesion-derived surface patterns can contain clinically relevant information [24].

AFM-based ML has also been applied to images of colorectal cancer cell lines with different neoplastic potential, indicating that topographical and physicochemical imaging channels can encode malignancy-related phenotypic information [21,26]. Together, these studies show that AI/ML can improve single-cell cancer classification by integrating stiffness, adhesion, surface organization, viscoelastic response, and image-derived features. For cytology-oriented applications, such approaches could complement conventional morphological assessment, although translation will require validation in primary patient-derived cells, standardized sample preparation, patient-level cross-validation, and evidence that AFM-derived classification adds value beyond existing cytological and molecular tests.

5.2. Tumor Tissue Classification and Nanomechanical Fingerprints

At the tissue level, AFM classification must account for the mechanical heterogeneity of solid tumors, which contain cancer cells, stromal cells, immune cells, blood vessels, ECM fibers, necrotic regions, and adipose or glandular compartments. Tumor progression is often associated with the coexistence of softer cell-rich regions and stiffer matrix-rich regions, producing broad or multimodal elasticity distributions that can serve as tissue-level nanomechanical fingerprints.

A landmark study in human breast biopsies showed that normal and benign tissues display relatively uniform stiffness profiles, whereas malignant tissues exhibit broader and more heterogeneous nanomechanical signatures reflecting distinct cellular and stromal compartments [52]. This concept has been further supported by later reviews and experimental studies, in which lower elasticity peaks were mainly associated with softer cell-rich regions and higher elasticity peaks or high-stiffness distributions with collagen-rich or ECM-dense compartments [7]. Similar disease-specific stiffness spectra and mechanical heterogeneity have been reported in liver, brain, cervix, oral submucous fibrosis, renal carcinoma, esophageal cancer, and colon cancer, supporting the potential of AFM-derived nanomechanical fingerprints as complementary biomarkers to histopathology, particularly when tumor margins, ambiguous morphology, or microenvironmental remodeling are clinically relevant.

AI/ML methods can strengthen tissue-level AFM classification by extracting information from complete stiffness distributions, force curves, elasticity maps, and multiparametric AFM images rather than relying on manually selected peaks or mean stiffness values. Distributional descriptors, spatial heterogeneity, texture features, and force-curve shapes can therefore be incorporated into automated models. For example, neural-network analysis of AFM force–distance curves has distinguished tumor from non-tumor brain tissues, highlighting the potential of ML to reduce curve-fitting burden and operator-dependent interpretation in tissue-level AFM studies [25].

5.3. Hepatocellular Carcinoma and Liver Disease

Hepatocellular carcinoma (HCC) is an important example of tissue-level AFM classification because liver cancer often develops within a mechanically remodeled background, such as fibrosis or cirrhosis. Although conventional diagnosis relies on imaging, histology, and molecular markers, AFM can provide complementary local mechanical information by detecting distinct nanomechanical signatures associated with HCC development and liver tissue composition [53].

Disease classification in liver tissue is complicated by the coexistence of tumor cells, fibrotic ECM, cirrhotic nodules, vascular structures, and inflammatory regions, which generate mechanically distinct compartments within the same sample. Complete stiffness distributions may therefore be more informative than mean stiffness values alone, as lower elasticity components may reflect cell-rich tumor regions, whereas higher stiffness

components may correspond to fibrotic or collagen-rich structures. AI/ML-assisted AFM workflows could integrate stiffness distributions, collagen-related image features, force-curve descriptors, and histological annotations to classify liver tissue states, distinguish malignant transformation from background fibrosis, identify high-risk regions, and support mechanical staging, although validation in independent patient cohorts and standardized biopsy-processing protocols remains necessary.

5.4. Brain Tumors

Brain tumors are highly relevant for AFM-based disease classification because nervous tissue is mechanically soft and sensitive to changes in ECM composition, cellular density, necrosis, and tumor infiltration. AFM studies have shown that human glioblastoma and meningothelial meningioma tissues exhibit distinct nanomechanical profiles, enabling differentiation of tumor core, necrotic regions, infiltrated regions, and adjacent tissue [93]. Such mechanical mapping may complement histopathology by identifying mechanically abnormal regions related to tumor type, grade, margin infiltration, and microenvironmental organization.

AI-assisted analysis may further support brain tumor classification by reducing dependence on manual curve fitting and operator interpretation. Neural-network-based analysis of AFM force–distance curves has been used to classify cancer tissues, including brain tumors, showing that raw or processed force-curve information can support automated diagnostic classification [25]. More recent AFM studies have also emphasized the value of combining nanomechanical mapping with morphological information [94], suggesting that future brain tumor assessment may benefit from multimodal integration of AFM mechanical maps with histology, optical imaging, Raman spectroscopy, or molecular markers.

5.5. Prostate, Colorectal, and Other Epithelial Cancers

AFM-based classification has also been explored in prostate, colorectal, and other epithelial cancers. Early single-cell AFM studies showed that metastatic cancer cells from patient samples can be softer than benign cells, supporting the potential of nanomechanical analysis to identify malignant or metastatic phenotypes [95]. In prostate cancer, AFM measurements comparing normal, benign, and malignant cells further indicated that changes in deformability can reflect disease state and metastatic potential, providing an important foundation for later AI-assisted mechanical classification.

Colorectal cancer is another promising area for AFM-based biomarker discovery, as disease progression involves cytoskeletal remodeling, altered adhesion, ECM interaction, and invasive potential. AFM studies have shown differences in surface morphology and mechanical properties between colorectal cancer cells with different aggressiveness, while ML analysis of AFM images has demonstrated that subtle topographical and mechanical features can distinguish closely related cancer cell phenotypes [21,26]. At the tissue level, colorectal tumors may also exhibit strong mechanical heterogeneity due to glandular architecture, stromal remodeling, desmoplasia, necrosis, and ECM deposition, suggesting that elasticity maps, adhesion patterns, and ML classification could support future tissue-level biomarker development.

Other epithelial malignancies, including cervical, oral, renal, esophageal, and bladder cancers, further illustrate the broad applicability of AFM-derived mechanical biomarkers. The strongest AI/ML evidence currently includes bladder cancer grading using multiparametric mechanical properties [12] and cervical cancer classification using adhesion map analysis [24]. Together, these studies suggest that AFM-based classification may be generalizable across malignancies, provided that disease-specific mechanical features are identified, standardized, and validated.

5.6. Fibrosis and Pulmonary Fibrosis

Fibrosis represents a major non-cancer application of AFM-derived nanomechanical biomarkers because disease progression is closely linked to ECM accumulation, collagen remodeling, altered tissue architecture, and progressive stiffening. Pulmonary fibrosis is particularly relevant, as alveolar remodeling, interstitial thickening, collagen deposition, and reduced lung compliance create measurable nanoscale and microscale mechanical changes.

AFM-based nanomechanical signatures have recently been proposed for pulmonary fibrosis staging and treatment monitoring. In human lung biopsies and bleomycin-induced murine models, AFM detected fibrotic-stage-dependent mechanical fingerprints that correlated with collagen content, histopathological staining, second harmonic generation microscopy, and gene-expression markers, while also enabling assessment of response to pirfenidone [9]. Although AI-assisted AFM studies in liver fibrosis, cardiac fibrosis, renal fibrosis, and systemic sclerosis remain limited, similar workflows could integrate stiffness distributions, collagen-related optical features, spatial mechanical descriptors, and ML-based classification to assess disease stage or treatment response across fibrotic disorders.

5.7. Other Non-Communicable Diseases

Beyond cancer and fibrosis, AFM has been applied to several mechanically active non-communicable diseases, but AI/ML-assisted AFM biomarker studies in these areas remain limited. In cardiovascular disease, AFM has been used to investigate vascular stiffness, endothelial mechanics, smooth muscle-cell behaviour, plaque composition, and ECM remodelling [96–98]. In musculoskeletal disorders, AFM stiffness mapping has characterized the pericellular and extracellular matrix of articular cartilage, supporting its relevance for osteoarthritis-related changes in cartilage mechanics and matrix organization [97,99,100]. AFM has also been applied to dystrophic muscle, where restoration of myofibre elasticity was measured after gene-mediated dystrophin rescue [101].

Neurodegenerative, diabetic, and wound-healing disorders represent additional emerging areas. In neurodegeneration, AFM can characterize the morphology, stiffness, and assembly of amyloid-β and tau aggregates, as well as neural cells and brain ECM components [102,103]. In diabetes and chronic wound-healing disorders, AFM may help detect nanoscale changes in collagen organization, ECM crosslinking, endothelial mechanics, tissue stiffness, and scar architecture [104–107]. However, most available studies in these disease areas are AFM biomechanics studies rather than direct AI/ML-assisted AFM classification studies. Therefore, these conditions should be viewed as emerging opportunities rather than mature applications of AI-enabled AFM biomarker discovery. Further disease-specific validation will be required before AI/ML-assisted AFM biomarkers can be considered translationally robust in these settings.

5.8. Current Limitations and Translational Perspectives in Disease Classification

Although AFM-derived nanomechanical biomarkers have shown promise for disease classification, the field remains at an early translational stage [108–110]. Many studies still rely on small datasets, limited patient numbers, or established cell lines, which increases the risk of overfitting and limits generalizability. In addition, experimental variability between laboratories, including sample preparation, hydration state, probe geometry, indentation depth, loading force, cantilever calibration, contact-model selection, and data-processing workflows, can strongly affect measured values. A further limitation is that classification performance is sometimes evaluated at the level of individual force curves, images, or cells rather than independent biological donors or patients, which can inflate accuracy if measurements from the same sample appear in both training and test datasets. Clinically

relevant validation will therefore require larger cohorts, patient-level data splitting, independent biological replicates, external validation, and standardized reporting protocols.

Future applications of AI-assisted AFM disease classification will be strongest where mechanical information provides added value beyond conventional diagnostic methods. In cancer, this may include tumor grading, margin assessment, identification of aggressive phenotypes, detection of precancerous-to-cancerous transition, and prediction of therapeutic response. In fibrosis, it may support disease staging, monitoring of antifibrotic therapy, and identification of mechanically active remodeling regions. Broader applications may include early detection of tissue degeneration, assessment of biomaterial integration, and quantification of ECM remodeling in other non-communicable diseases. As summarized in Figure 3, AFM-derived readouts such as stiffness, adhesion, viscoelasticity, mechanical heterogeneity, and topographical features can be integrated with AI/ML analysis to classify cancer cells, tumor tissues, hepatocellular carcinoma, brain tumors, prostate/colorectal cancers, pulmonary and liver fibrosis, cardiovascular diseases, neurodegenerative diseases, and musculoskeletal disorders.

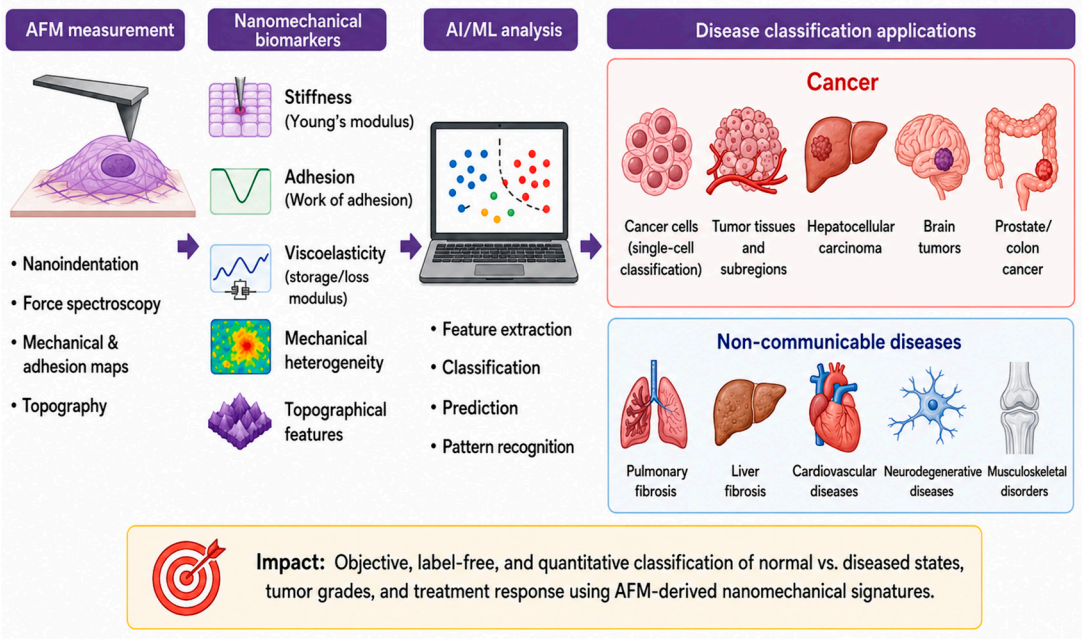


Figure 3. Applications of AFM-derived nanomechanical biomarkers in disease classification. AFM measurements generate multiparametric nanomechanical readouts, including stiffness, adhesion, viscoelasticity, mechanical heterogeneity, and topographical features, which can be integrated with AI/ML analysis for disease classification. These approaches have been applied to cancer cells and tumour tissues, including hepatocellular carcinoma, brain tumours, and prostate/colorectal cancers, as well as non-communicable diseases such as pulmonary and liver fibrosis, cardiovascular diseases, neurodegenerative diseases, and musculoskeletal disorders. Together, these applications highlight the potential of AFM-derived nanomechanical fingerprints as objective and quantitative biomarkers for distinguishing normal and pathological states, tumour grades, and treatment response patterns.

To move toward clinical translation, future studies should shift from single-parameter analysis toward multiparametric and multimodal models that combine stiffness distributions, adhesion maps, viscoelastic properties, roughness descriptors, force-curve features, and mechanical heterogeneity with histopathology, collagen imaging, fluorescence microscopy, second harmonic generation microscopy, Raman spectroscopy, molecular biomarkers, and clinical metadata. Explainable AI will also be essential, as clinically useful models should not only classify samples but also reveal whether predictions are driven by biologically meaningful features such as stiffness heterogeneity, adhesion patterns,

collagen-associated stiffening, roughness, or force-curve shape rather than artifacts related to scan noise, sample preparation, or instrument-specific signatures. Overall, AI/ML can strengthen AFM-based disease classification by enabling objective, automated, and interpretable analysis, but translation will require standardized protocols, larger datasets, patient-level validation, external reproducibility, and evidence that AFM-derived mechanical information improves diagnosis, staging, prognosis, or treatment monitoring beyond existing approaches.

6. Applications in Treatment Monitoring and Prognosis

AFM-derived nanomechanical biomarkers are not limited to disease detection or classification, but may also support treatment monitoring and prognosis. Many therapies induce early structural and mechanical changes in cells, tissues, and ECM before macroscopic tumor shrinkage, radiological improvement, or histopathological remodeling becomes evident. In cancer, these changes may reflect tumour-cell death, reduced proliferation, cytoskeletal disruption, ECM degradation, stromal normalization, therapy-induced fibrosis, or altered mechanical heterogeneity, whereas in fibrotic disease they may involve reduced collagen deposition, ECM reorganization, decreased tissue stiffness, and partial restoration of a more physiological mechanical microenvironment. Because AFM can quantify local cellular and tissue mechanics at nanoscale and microscale resolution, AFM-derived stiffness distributions, adhesion patterns, force-curve descriptors, viscoelastic parameters, and spatial heterogeneity, especially when combined with AI/ML, could provide sensitive and quantitative readouts for therapeutic response, prognosis, and personalized treatment strategies [8,9].

6.1. Therapy-Induced Stiffness Changes as Early Response Biomarkers

One of the most direct applications of AFM in treatment monitoring is the detection of therapy-induced stiffness changes. At the cellular level, drugs can alter cytoskeletal organization, actomyosin contractility, membrane tension, adhesion, and viscoelasticity. These alterations can be detected by AFM as changes in Young's modulus, adhesion force, work of adhesion, deformation, hysteresis, or relaxation behavior. Depending on the drug mechanism and cell type, therapy may induce either softening or stiffening. For example, cytoskeletal-disrupting agents may reduce cell stiffness, whereas apoptosis, increased cortical tension, or treatment-induced stress responses may increase stiffness. Therefore, the direction of stiffness change is not universal; rather, the mechanical response must be interpreted in relation to the treatment mechanism, cell phenotype, and microenvironmental context.

At the tissue level, treatment-induced stiffness changes can be even more complex. A therapy may kill tumour cells but leave behind a dense ECM network, leading to increased apparent tissue stiffness. Conversely, therapies that remodel the tumor microenvironment may reduce collagen content, decompress blood vessels, and decrease the high-stiffness component of the tissue. Therefore, treatment monitoring should not rely only on mean stiffness. Instead, the entire stiffness distribution, including lower and higher elasticity peaks, distribution width, multimodality, and spatial heterogeneity, may provide a more complete picture of therapeutic response [7,8].

This concept is central to the use of AFM as a treatment-monitoring platform. A responsive tumor or fibrotic tissue may show a shift in the elasticity spectrum, a reduction in mechanically stiff regions, a change in the ratio between soft and stiff components, or a decrease in mechanical heterogeneity. AI and ML methods can be used to detect such changes more sensitively than conventional statistical comparison of mean stiffness values. For example, ML models can incorporate distributional descriptors, force-curve shapes,

adhesion features, and spatial patterns to classify treated versus untreated tissues or to predict which samples are likely to respond to therapy.

6.2. Tumor Microenvironment Normalization and Mechanotherapeutics

Tumor progression is strongly influenced by the mechanical properties of the tumor microenvironment (TME). Many solid tumors are characterized by excessive ECM deposition, collagen crosslinking, hyaluronan accumulation, stromal-cell activation, elevated solid stress, and vessel compression. These mechanical abnormalities can impair perfusion, limit drug delivery, promote hypoxia, and reduce therapeutic efficacy. Therefore, therapies that normalize the mechanical microenvironment have emerged as promising strategies to improve drug delivery and treatment response. Such strategies include antifibrotic agents, anti-stromal therapies, matrix-remodeling approaches, antihypertensive drugs, and agents that reprogram cancer-associated fibroblasts [111,112].

AFM is well suited to monitor TME normalization because the therapeutic target is, at least in part, mechanical. In tumor models treated with the mechanotherapeutic agent tranilast, AFM measurements were able to detect changes in tumor nanomechanical properties during therapy. These changes correlated with alterations in ECM components, including collagen and hyaluronan, and reflected the ability of normalization treatment to remodel the tumor mechanical microenvironment [8]. Importantly, such measurements showed that AFM can detect treatment-induced nanomechanical changes in both breast cancer and fibrosarcoma models, supporting the idea that AFM-derived elasticity spectra can act as monitoring biomarkers for mechanotherapeutic interventions.

The clinical relevance of this approach is significant. If AFM can identify whether a tumor is mechanically normalized after treatment, it could help determine the optimal timing for chemotherapy, nanomedicine, immunotherapy, or photodynamic therapy. Since normalization effects may be transient, a sensitive mechanical biomarker could assist in selecting the therapeutic window during which drug delivery and immune-cell infiltration are improved. AI-enabled AFM analysis could further support this goal by detecting subtle distributional shifts, identifying mechanically responsive tumor subregions, and integrating AFM data with vascular, collagen, and molecular readouts.

6.3. Chemotherapy-Induced Mechanical Remodeling

Chemotherapy can alter cell and tissue mechanics through cytoskeletal disruption, cell death and ECM remodeling. AFM may detect these changes as shifts in stiffness distributions, adhesion or mechanical heterogeneity before macroscopic responses become evident. AFM-based monitoring has been demonstrated in tumor models treated with doxorubicin alone or in combination with TME normalization therapy, where stiffness distributions captured nanomechanical changes associated with therapeutic response [8].

This approach is particularly relevant when chemotherapy is combined with mechanotherapeutics, since stiff and poorly perfused tumors can limit drug delivery, whereas TME normalization may reduce mechanical barriers, improve vascular function, and enhance drug penetration. AFM-derived mechanical biomarkers could therefore help monitor whether treatment shifts the tumor toward a more permissive mechanical state. From an AI/ML perspective, chemotherapy-response monitoring could involve classification of untreated, partially responsive, and responsive tumors, longitudinal tracking of mechanical trajectories, and identification of baseline AFM-derived features predictive of later therapeutic response.

6.4. Immunotherapy and Mechanical Biomarkers

Cancer immunotherapy has transformed oncology, but responses remain variable across tumor types and patients. The mechanical tumor microenvironment can influence

immunotherapy response by regulating vascular perfusion, immune-cell infiltration, T-cell migration, stromal barriers, and mechanotransduction pathways [111,112]. Dense collagen networks, elevated stiffness, compressed vessels, and solid stress may restrict immune-cell access and contribute to immunosuppression, suggesting that mechanical biomarkers could help explain differences between immunotherapy-sensitive and resistant tumors.

Several studies support the therapeutic relevance of targeting mechanical or stromal components of the TME. Endothelin inhibition was shown to potentiate cancer immunotherapy and reveal mechanical biomarkers predictive of response [112], while tumor microenvironment reprogramming using polymeric micelles enhanced nano-immunotherapy in breast cancer models [111]. More recently, stabilization of tumor-resident mast cells restored T-cell infiltration and sensitized sarcomas to PD-L1 inhibition, further linking stromal organization, immune access, and therapeutic response [113].

AFM-derived nanomechanical biomarkers could contribute by identifying mechanical profiles associated with immunotherapy sensitivity or resistance, such as high stiffness, collagen-associated elasticity peaks, or pronounced mechanical heterogeneity. Because immunotherapy response depends on interactions among tumor cells, ECM, vasculature, immune cells, and stromal components, AI/ML models could integrate AFM-derived stiffness distributions, collagen-related image features, immune-cell infiltration markers, vascular functionality, and molecular data to identify mechanical phenotypes associated with immune exclusion, immune infiltration, or checkpoint-blockade sensitivity.

6.5. Photodynamic Therapy and Mechano-Biophysical Monitoring

Photodynamic therapy (PDT) relies on light activation of a photosensitizer in the presence of oxygen to generate reactive oxygen species and induce cell death. Its efficacy depends on photosensitizer uptake, oxygen availability, vascular function, ECM organization, tissue optical properties, and tumor architecture, many of which are influenced by the physical and mechanical state of the tumor. Mechanical abnormalities such as ECM stiffening, collagen accumulation, and vessel compression may restrict photosensitizer penetration and oxygen delivery, supporting the rationale for combining PDT with TME-targeting or normalization strategies [114].

AFM-derived biomarkers could contribute to PDT optimization by assessing baseline tumor stiffness and heterogeneity, monitoring PDT-induced changes in cancer cell mechanics, adhesion, membrane integrity, and cytoskeletal organization, and detecting post-treatment ECM or stromal remodeling. When combined with AI/ML, these measurements could help identify mechanical signatures associated with photosensitizer efficacy, oxidative damage, or treatment resistance. In tissue models, shifts in AFM elasticity spectra could be integrated with collagen density, hypoxia markers, light penetration, and treatment outcomes to support more personalized PDT protocols.

6.6. Antifibrotic Treatment Monitoring

Fibrotic diseases are particularly suitable for AFM-based treatment monitoring because their progression and therapeutic response are directly linked to ECM remodeling and tissue stiffness. In pulmonary fibrosis, excessive deposition of collagen and other ECM components leads to tissue stiffening, impaired lung architecture, and progressive loss of function. Current antifibrotic therapies, such as pirfenidone and nintedanib, can slow disease progression but show variable effectiveness across patients. Reliable biomarkers for staging and treatment monitoring remain urgently needed.

AFM-based nanomechanical signatures have recently been used to monitor pulmonary fibrosis progression and response to pirfenidone treatment. In human lung biopsies and bleomycin-induced murine models, AFM detected distinct nanomechanical finger-

prints associated with fibrosis stage. These fingerprints correlated with collagen I content, histopathological staining, second harmonic generation microscopy, and gene expression. Importantly, AFM-derived measurements were able to assess changes after pirfenidone treatment, supporting the use of nanomechanical fingerprints as biomarkers for antifibrotic therapy response [9].

The significance of this application extends beyond pulmonary fibrosis. Many chronic non-communicable diseases involve fibrotic remodeling, including liver fibrosis, cardiac fibrosis, renal fibrosis, systemic sclerosis, and diabetes-associated tissue stiffening. In all these conditions, treatment response may involve changes in ECM deposition, collagen organization, tissue hydration, crosslinking, and local mechanical heterogeneity. AFM can capture these alterations at length scales not accessible by conventional elastography or histology alone.

AI/ML can strengthen antifibrotic treatment monitoring by integrating stiffness distributions with collagen imaging, histological scores, and molecular markers. For example, a model could classify tissue as healthy, early fibrotic, advanced fibrotic, treated-responsive, or treated-nonresponsive based on AFM-derived mechanical descriptors. Longitudinal mechanical profiling could also reveal whether treatment prevents progression, reverses stiffness, or modifies only specific tissue compartments. Such approaches may eventually support individualized antifibrotic therapy selection.

6.7. Prognostic Value of AFM-Derived Mechanical Signatures

Beyond monitoring response to specific therapies, AFM-derived nanomechanical biomarkers may also have prognostic value. In cancer, mechanical properties are linked to invasion, metastatic potential, drug delivery, immune exclusion, and treatment resistance. Tumors with high ECM stiffness, pronounced mechanical heterogeneity, or strong stromal mechanical signatures may be more aggressive or less responsive to therapy, whereas mechanically normalized tumors may show improved perfusion, reduced stromal barriers, and better treatment outcomes. At the single-cell level, increased deformability, altered adhesion, and changes in membrane tension may reflect migratory or metastatic potential, while at the tissue level, high stiffness and broad elasticity distributions may indicate desmoplasia, collagen accumulation, or stromal activation.

In fibrotic diseases, increased stiffness and persistent mechanical heterogeneity may similarly reflect advanced disease and poor treatment response. Therefore, AFM-derived parameters could potentially help predict disease progression, recurrence risk, metastatic behavior, or response to therapy. Because prognosis is unlikely to depend on a single mechanical variable, AI/ML models will be important for integrating AFM-derived stiffness distributions, adhesion features, viscoelasticity, histological scores, collagen organization, immune infiltration, gene expression, and clinical outcome data. Such multimodal models could eventually identify mechanical signatures associated with progression-free survival, overall survival, recurrence, or therapeutic response, although this will require large longitudinal datasets and rigorous validation.

6.8. Challenges for Treatment-Monitoring Applications

Although AFM-based treatment monitoring is promising, several challenges must be addressed. First, therapy-induced mechanical changes may be time-dependent and transient. The timing of biopsy or measurement relative to treatment is therefore critical. Second, mechanical changes can be spatially heterogeneous; sampling only one region may not represent the entire tumor or fibrotic organ. Third, treatment may affect different tissue compartments in opposite ways. For example, cancer cell death may reduce cellular

stiffness while residual ECM may remain stiff, or stromal remodeling may reduce stiffness while inflammatory infiltration alters local mechanics.

Fourth, AFM measurements are sensitive to sample preparation, hydration, temperature, probe calibration, indentation depth, and analysis model. These factors must be standardized, especially when comparing pre- and post-treatment samples. Fifth, longitudinal clinical monitoring with AFM may be limited by biopsy availability. Therefore, AFM may initially be most useful in ex vivo biopsy analysis, preclinical models, organoids, patient-derived explants, and mechanistic treatment studies.

AI/ML models also require careful validation. Treatment-monitoring datasets are often small, and repeated measures from the same animal, patient, or tumor can create data-dependence issues. Models should be validated using independent biological replicates and, ideally, independent cohorts. Moreover, treatment labels alone are not sufficient; models should be trained and tested against meaningful outcomes, such as histological response, collagen reduction, tumor growth delay, improved drug delivery, immune infiltration, or survival.

6.9. Future Perspectives

The future of AFM-derived biomarkers in treatment monitoring will likely involve integrated, multiparametric, and multimodal workflows. Rather than measuring stiffness alone, AFM studies should incorporate adhesion, viscoelasticity, roughness, force-curve descriptors, stiffness distributions, and mechanical heterogeneity. These features should be correlated with histopathology, collagen imaging, vascular function, immune profiling, molecular biomarkers, and therapeutic outcomes. AI and ML can then be used to identify mechanical signatures that predict or monitor response.

In oncology, AFM-based monitoring may help optimize chemotherapy, immunotherapy, nanomedicine, PDT, and TME normalization strategies. In fibrosis, it may help stage disease and evaluate antifibrotic treatment response. In both contexts, the most clinically useful biomarkers may not be absolute stiffness values but changes in mechanical fingerprints over time. Such changes could indicate whether a tissue is becoming more normalized, more fibrotic, more heterogeneous, or more resistant to therapy.

In summary, AFM-derived nanomechanical biomarkers have strong potential for treatment monitoring and prognosis because many therapies induce measurable changes in cell and tissue mechanics. Evidence from tumor microenvironment normalization, chemotherapy, immunotherapy-related mechanobiology, photodynamic therapy concepts, and antifibrotic treatment supports the idea that mechanical fingerprints can provide valuable therapeutic information. The integration of AFM with AI/ML will be essential for converting these complex mechanical datasets into predictive, reproducible, and clinically meaningful biomarkers.

7. Automated and High-Throughput AFM Mechanomics

Although AFM provides unique access to nanoscale mechanical and physicochemical properties of cells and tissues, its broader use as a biomarker platform is restricted by limited throughput, operator dependence, and time-consuming acquisition and analysis. Conventional AFM experiments often require manual selection of regions of interest, careful probe positioning, repeated calibration, visual assessment of image quality, and post-acquisition processing of thousands of force curves. These requirements are manageable in focused mechanobiology studies but become major barriers when AFM is proposed for large-scale biomarker discovery, patient stratification, treatment monitoring, or clinical translation.

The concept of AFM mechanomics has emerged from the need to move beyond isolated measurements toward high-content, multiparametric, and scalable mechanical

phenotyping. In this context, mechanomics refers to the systematic acquisition and computational analysis of mechanical, adhesive, viscoelastic, topographical, and spatial features from cells, tissues, or biomaterials. Similar to genomics or proteomics, the goal is not only to measure one parameter, such as Young's modulus, but to define complex mechanical signatures that reflect biological state, disease progression, or treatment response. AI and ML are central to this transition because they can automate several steps of the AFM workflow and extract meaningful patterns from large and heterogeneous datasets [17,19].

7.1. Automation of AFM Acquisition

A major limitation of traditional AFM is the need for continuous user supervision. The operator typically selects the scan area, positions the probe, evaluates whether the sample surface is suitable, adjusts imaging parameters, and decides whether the acquired data are acceptable. This introduces subjectivity and limits throughput. ML-guided automation can assist in selecting appropriate scanning regions, detecting cells or tissue structures, identifying surface artifacts, and navigating the probe between relevant locations. Such automation is especially important for biological samples, where cells vary in shape, size, height, adhesion, and mechanical state.

Automated region-of-interest selection is particularly valuable for single-cell mechanophenotyping. In conventional workflows, the operator must identify cells manually under an optical microscope or AFM image, move the probe to the desired location, and perform indentation or mapping. This process is slow and may introduce selection bias. ML-based image recognition can identify cell boundaries, classify cell morphology, detect suitable indentation sites, and guide the AFM probe toward predefined cellular regions. Such approaches can reduce operator intervention and improve reproducibility, especially when large numbers of cells must be measured for statistically meaningful classification.

AFM automation can also improve tissue-level analysis. Tissue biopsies are mechanically heterogeneous, and the choice of measurement region can strongly influence the resulting elasticity spectrum. Automated workflows could sample multiple regions systematically, avoid damaged or folded areas, and collect force maps from both cell-rich and ECM-rich regions. In the future, ML-assisted AFM could be combined with optical microscopy, fluorescence imaging, or histological pre-screening to direct measurements toward specific tissue compartments. This would allow mechanical information to be spatially linked with biological structure.

7.2. High-Speed and High-Throughput AFM Modes

High-throughput AFM requires both faster acquisition and more efficient analysis. Several advanced AFM modes, including PeakForce tapping, quantitative nanomechanical mapping, off-resonance tapping, high-speed AFM, multifrequency AFM, and force-volume automation, have been developed to increase acquisition speed and generate multiparametric maps. These modes can provide topography, adhesion, deformation, dissipation, and mechanical-property maps more rapidly than classical point-by-point force spectroscopy. However, faster acquisition often involves trade-offs in indentation depth, force control, signal-to-noise ratio, and suitability for very soft biological samples.

High-speed nanomechanical mapping is promising for biomarker studies because disease classification frequently requires many measurements across many cells or tissue regions. For example, tumor biopsies and fibrotic tissues are highly heterogeneous, and small numbers of force curves may not adequately represent the mechanical phenotype. Larger datasets can improve the statistical robustness of stiffness distributions and allow ML models to detect subtle differences between disease stages or treatment groups. However,

high-throughput acquisition also produces large datasets that are difficult to analyze manually. Thus, faster AFM modes require AI-enabled postprocessing to become practical.

In the context of single-cell studies, high-throughput mechanophenotyping may allow AFM to approach the scale needed for cytology-like applications. Instead of measuring a few cells per condition, automated AFM could measure hundreds or thousands of cells, capturing population-level mechanical heterogeneity. This is important because disease states often involve mechanical subpopulation rather than uniform changes across all cells. ML models could then classify cells based on multiparametric mechanical profiles and identify rare but clinically relevant phenotypes, such as highly invasive, treatment-resistant, or metastatic subpopulations.

7.3. Automated Force-Curve Processing and Quality Control

Even with automated acquisition, force-curve analysis remains a major bottleneck because AFM force spectroscopy can generate thousands to millions of curves requiring baseline correction, contact-point detection, indentation calculation, model fitting, quality control, and parameter extraction. Manual or semi-automated processing is slow and can introduce user-dependent variability, whereas AI/ML methods can support automated artifact detection, poor-curve rejection, contact-point estimation, fitting-region selection, and direct prediction of mechanical properties from curve shape.

Automated curve-quality control is essential because unreliable curves caused by probe contamination, sample movement, surface roughness, excessive noise, incorrect contact detection, tissue detachment, or unsuitable indentation regions can distort stiffness distributions and reduce classification accuracy. ML-based quality-control models could identify reliable curves using curve morphology, noise structure, fitting residuals, adhesion behavior, and consistency with neighboring measurements.

Deep learning and data-driven regression approaches may further reduce dependence on traditional contact-model fitting. Neural-network regressors trained on synthetic force–indentation curves generated from contact mechanics models have been shown to predict Young's modulus and adhesion energy from AFM nanoindentation data [13]. If carefully validated in biological samples, such approaches could accelerate post-processing and convert large raw-curve datasets into standardized descriptors, including stiffness distributions, adhesion metrics, viscoelastic parameters, and quality-controlled mechanical maps for disease classification, staging, or treatment monitoring.

7.4. Multiparametric Mechanomics and Data Integration

The full potential of high-throughput AFM lies in multiparametric mechanomics. Each AFM experiment can generate several complementary data streams: topography, stiffness, adhesion, deformation, dissipation, roughness, viscoelasticity, force-curve descriptors, and spatial heterogeneity. When these parameters are analyzed together, they may provide a richer description of biological state than any single parameter alone. ML methods are particularly well suited for integrating such multidimensional data.

In cancer cell classification, multiparametric AFM analysis has already shown advantages over single-feature approaches. For example, the integration of elastic modulus, work of adhesion, adhesiveness, and membrane tension improved the classification of bladder cancer cells at different grades [12]. Similarly, AFM adhesion map analysis combined with ML improved the discrimination between precancerous and cancerous cervical epithelial cells [24]. These examples show that AI-enabled mechanomics can reveal diagnostic information distributed across multiple mechanical and surface features.

At the tissue level, multiparametric mechanomics could integrate stiffness spectra, elasticity-map texture, adhesion patterns, collagen-related stiffness, and topographical

features. In tumor tissues, this may help distinguish cell-rich regions, ECM-rich regions, necrotic areas, stromal compartments, and mechanically normalized regions after therapy. In pulmonary fibrosis, it may help classify disease stage by combining stiffness distributions with collagen organization, histology, and second harmonic generation microscopy [9]. Such multimodal integration may be essential for developing clinically meaningful biomarkers.

7.5. Toward Autonomous AFM Biomarker Platforms

A long-term goal is the development of autonomous AFM biomarker platforms that integrate standardized acquisition, ML-guided scanning, automated probe calibration, real-time image assessment, force-curve quality control, multiparametric feature extraction, and AI-based classification with minimal operator intervention. As illustrated in Figure 4, such platforms could combine autonomous region selection, high-throughput AFM mapping, automated force-curve processing, large mechanome dataset generation, and AI/ML analysis to produce clinically interpretable mechanomic signatures rather than isolated mechanical values. In cancer, this could support mechanical phenotyping of cells from minimally invasive samples, detection of suspicious populations, mapping of disease-associated stiffness patterns in biopsies, and comparison of baseline and post-treatment nanomechanical signatures. In fibrosis, similar workflows could classify disease stage and quantify response to antifibrotic therapy.

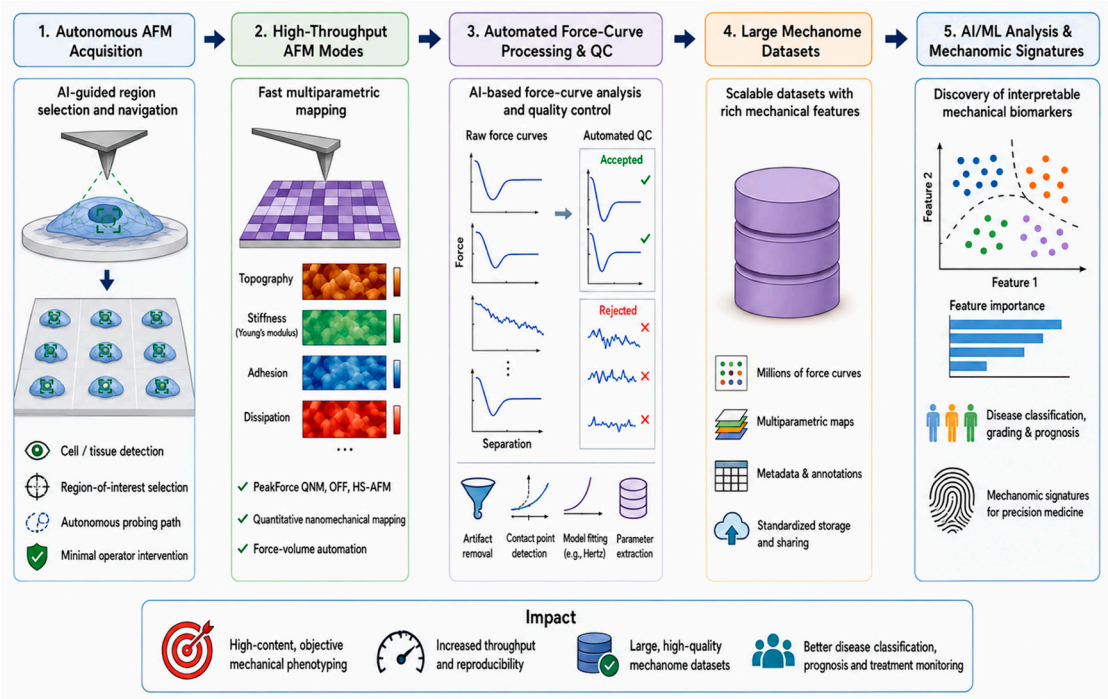


Figure 4. Automated and high-throughput AFM mechanomics workflow. AI-assisted AFM mechanomics enables scalable and reproducible nanomechanical phenotyping by integrating autonomous AFM acquisition, high-throughput mapping modes, automated force-curve processing, quality control, and large mechanome dataset generation. These standardized multiparametric datasets can then be analyzed using AI/ML approaches to extract interpretable mechanomic signatures for disease classification, grading, prognosis, treatment monitoring, and precision mechanobiology.

However, autonomous AFM systems must be robust across sample types, instruments, probes, operators, and laboratories, while also handling biological variability, tissue heterogeneity, and technical artifacts. Real-time AI decision-making should be transparent, externally validated, and interpreted alongside histopathology, molecular assays, imag-

ing, and clinical data rather than replacing biological interpretation. Overall, automated and high-throughput AFM mechanomics represents a critical step toward translating AFM-derived nanomechanical biomarkers into scalable platforms for mechanobiological phenotyping, disease classification, grading, prognosis, treatment monitoring, and precision mechanobiology.

8. Data Standards and Reproducibility

The translation of AI-enabled AFM nanomechanical biomarkers requires not only advanced algorithms but also standardized data acquisition, processing, reporting, and validation. AFM measurements are highly sensitive to experimental conditions, and ML models are highly sensitive to the quality and structure of the data used for training. Therefore, reproducibility is a central requirement for the field. Without standardized protocols and transparent reporting, AFM-derived biomarkers may remain difficult to compare across studies, instruments, laboratories, and disease models.

Reproducibility challenges arise at several levels. First, AFM measurements depend on probe geometry, spring constant calibration, deflection sensitivity, indentation depth, loading rate, environmental conditions, and sample preparation. Second, data analysis depends on contact-point detection, curve preprocessing, fitting range, contact mechanics model, Poisson's ratio, and exclusion criteria. Third, ML analysis depends on feature extraction, data splitting, normalization, model selection, hyperparameter tuning, cross-validation, and statistical testing. Variability at any of these levels can affect the final biomarker signature.

8.1. Standardization of AFM Acquisition

Standardized acquisition protocols are essential for comparing AFM-derived mechanical values across studies. Key experimental parameters should be reported in detail, including AFM mode, probe type and geometry, tip radius, cantilever spring constant, calibration method, deflection sensitivity, maximum force, indentation depth, loading and unloading rates, dwell time, scan size, number of curves, force-map resolution, sample temperature, measurement medium, hydration state, and time between sample collection and measurement.

Major sources of variability include probe calibration, probe geometry, and sample preparation. Errors in cantilever spring constant or deflection sensitivity directly affect force calculation and propagate into Young's modulus and adhesion estimates, making standardized calibration procedures and reference materials important for reproducibility. Probe geometry should also be clearly documented because sharp tips, conical or pyramidal probes, and spherical or colloidal probes differ in spatial resolution, contact area, model suitability, and apparent modulus values. Similarly, biological samples may be measured live, fixed, frozen, fresh, embedded, sectioned, or hydrated under different conditions, each of which can alter mechanical properties. For clinical translation, AFM protocols must therefore be compatible with realistic biopsy workflows while preserving mechanical integrity.

8.2. Standardization of Data Processing

AFM data processing can strongly influence extracted biomarker values, particularly in force spectroscopy, where contact-point determination remains a major source of uncertainty for soft, rough, adhesive, or heterogeneous biological samples. Baseline correction, noise filtering, indentation calculation, curve truncation, fitting-region selection, and contact-model choice can all affect Young's modulus, adhesion, and related parameters. Hertz and Sneddon models are widely used but assume elastic, homogeneous, isotropic,

non-adhesive, and infinitely thick samples, whereas adhesive, viscoelastic, or poroelastic models may be more appropriate when these assumptions are violated. Therefore, preprocessing steps, model selection, software settings, and analysis scripts should be reported transparently, and model choice should be justified and consistently applied.

For biomarker studies, the main goal is often classification rather than determination of an absolute material constant, but consistency in analysis remains essential. If groups are processed differently, apparent classification may reflect analytical artifacts rather than biological differences. Preprocessing pipelines should therefore be fixed before model training, applied identically to all groups, and performed within the cross-validation workflow when ML models are used to avoid data leakage. Quality-control criteria should also be standardized, including rules for excluding curves with poor contact, excessive noise, non-physical shape, poor fitting quality, sample drift, or probe contamination. Automated quality-control algorithms may improve reproducibility, but they must be clearly described and validated.

8.3. Reporting of AFM-Derived Biomarker Features

AFM biomarker studies should report not only mean or median Young's modulus but also distributional and spatial descriptors when relevant, since biological tissues are heterogeneous and stiffness distributions may contain disease- or treatment-related information. Key reporting parameters should include sample size, number of biological and technical replicates, number of measured cells or tissue regions, number of force curves, distribution shape, variance, percentile values, and peak positions. For tissue-level studies, elasticity spectra should be presented with representative mechanical maps and, ideally, correlated with histology or structural imaging; when lower or higher elasticity peaks or mechanical heterogeneity metrics are reported, the peak-detection method and heterogeneity definition should be clearly described.

In ML-based AFM studies, the full feature set should be reported, including whether features were extracted from raw force curves, fitted parameters, images, maps, histograms, or manually engineered descriptors. For AFM image-based ML, the imaging channels should be specified, such as height, adhesion, deformation, dissipation, stiffness, roughness, or related maps. Because some AFM channels are more instrument-dependent than others, repeatability should be considered, and physically meaningful channels such as height, adhesion, and deformation may be more suitable for cross-laboratory ML analysis than strongly instrument-dependent error or phase signals [21].

8.4. Data Standards for AI/ML Analysis

AI/ML-based AFM studies introduce additional reproducibility requirements, including transparent reporting of data preprocessing, normalization, feature scaling, dimensionality reduction, feature selection, model type, hyperparameters, training strategy, validation method, and performance metrics. The unit of data splitting should match the biological question; for example, patient-level diagnosis requires splitting by patient rather than by force curve, image, or cell. If measurements from the same biopsy, cell, animal, or patient appear in both training and test sets, model performance may be artificially inflated by sample-specific or batch-specific features. For translational studies, grouped cross-validation, leave-one-patient-out validation, leave-one-sample-out validation, or independent external test cohorts are therefore more appropriate than random curve-level splitting.

Performance reporting should extend beyond accuracy and include sensitivity, specificity, precision, recall, F1-score, area under the receiver operating characteristic curve, confusion matrices, calibration, and confidence intervals. This is particularly important for

imbalanced datasets, diagnostic applications, and treatment response prediction, where sensitivity, specificity, and predictive values may be more clinically informative than overall accuracy. Permutation testing or random-label shuffling should also be used to assess statistical significance, especially in small AFM datasets, where ML models can overfit and produce apparently strong but poorly reproducible results [21].

8.5. Avoiding Overfitting and Data Leakage

Overfitting and data leakage are major risks in AI-enabled AFM biomarker studies because datasets often contain many features but relatively few independent biological samples. Models may perform well during training but fail on new samples, especially when feature selection, dimensionality reduction, normalization, or hyperparameter tuning is performed before data splitting. To avoid this, all model-selection and preprocessing steps should be performed only within the training set or within nested cross-validation, ensuring that information from the test set does not influence model development.

Batch effects should also be controlled because AFM data can be influenced by measurement day, operator, instrument, probe batch, culture passage, sample preparation, and storage conditions. If experimental groups are measured under different technical conditions, ML models may learn batch-specific differences rather than disease biology. Measurements should therefore be randomized across groups, batch variables should be recorded, and, when possible, models should be tested on data collected on different days, instruments, or laboratories.

8.6. Open Data, Shared Benchmarks, and FAIR Principles

The field would benefit substantially from open AFM datasets and shared benchmark tasks. Publicly available datasets would allow independent validation of algorithms, comparison of ML methods, development of standardized preprocessing pipelines, and assessment of reproducibility across laboratories. At present, many AFM-ML studies use private datasets, making it difficult to compare performance or reproduce results.

AFM datasets should follow FAIR principles: findable, accessible, interoperable, and reusable. This requires standardized metadata, including sample type, disease state, preparation protocol, instrument, probe, acquisition parameters, calibration details, environmental conditions, preprocessing steps, and biological annotations. Raw force curves, processed maps, extracted features, and labels should be stored in formats that can be reused by other researchers. Open-source code for analysis and ML workflows should be provided whenever possible.

Shared benchmarks could include tasks such as force-curve quality classification, Young's modulus prediction, cancer cell classification, adhesion map classification, fibrosis-stage classification, and treatment response prediction. These benchmarks would help determine which algorithms perform robustly across datasets and which features are most reproducible.

8.7. Biological Validation and Multimodal Correlation

Reproducibility is not only technical; AFM-derived biomarkers must also be biologically validated. Mechanical features should be correlated with independent biological readouts such as histology, immunohistochemistry, collagen staining, second harmonic generation microscopy, gene expression, cytoskeletal markers, vascular function, immune infiltration, or clinical outcome. Without biological validation, a mechanical signature may classify samples but remain difficult to interpret.

For tumor tissues, AFM stiffness distributions should be related to collagen content, stromal organization, cellular density, necrosis, and vascular structure. For pulmonary fibrosis, nanomechanical fingerprints should be correlated with collagen I expression, histologi-

cal fibrosis scores, SHG imaging, and response to antifibrotic treatment [9]. For cell-based studies, stiffness and adhesion features should be linked to cytoskeletal organization, membrane composition, metastatic potential, or drug response. Such correlations strengthen the argument that AFM-derived biomarkers reflect meaningful disease mechanisms.

Multimodal integration will likely be essential for clinical translation. AFM alone may not replace histopathology, molecular diagnostics, or imaging, but it can provide complementary mechanical information. AI/ML methods can integrate AFM-derived features with other modalities to build more robust and biologically interpretable classifiers. For example, a fibrosis classifier could combine AFM stiffness distributions with SHG collagen features and gene-expression markers. A cancer classifier could combine AFM mechanical heterogeneity with histological annotations and immune markers.

8.8. Toward Reproducible AI-Enabled AFM Biomarkers

For AI-enabled AFM biomarkers to become reliable, the field should move toward standardized reporting guidelines. Such guidelines should include minimum information on sample preparation, AFM acquisition, calibration, data processing, feature extraction, ML workflow, validation strategy, and biological interpretation. Similar to reporting standards in genomics, imaging, and clinical prediction modelling, AFM biomarker studies would benefit from structured checklists.

A reproducible AFM biomarker study should include: clearly defined biological question; adequate biological replication; standardized sample preparation; detailed AFM acquisition parameters; calibration and quality-control procedures; transparent preprocessing; predefined feature extraction; appropriate model validation; patient- or sample-level data splitting; reporting of multiple performance metrics; permutation or statistical significance testing; explainability analysis; and biological validation.

In summary, data standards and reproducibility are central to the future of AFM-derived nanomechanical biomarkers. AI and ML can greatly enhance AFM analysis, but they cannot compensate for poorly standardized acquisition, biased datasets, or insufficient validation. The successful translation of AI-enabled AFM mechanobiomarkers will require coordinated advances in experimental protocols, data infrastructure, open benchmarking, statistical rigor, explainable modelling, and multimodal biological validation.

9. Conclusions and Future Perspectives

This review highlights that AI and ML are beginning to transform AFM-based nanomechanical biomarker research from single-parameter stiffness analysis toward multiparametric, automated, and more interpretable analysis of cells, tissues, and extracellular matrix. Published studies have shown that AI/ML can support cancer cell classification, adhesion map analysis, tissue recognition, force-curve interpretation, fibrosis staging, prediction of mechanical properties, and treatment response monitoring [8,9,12,13,21,24]. These contributions are important because AFM datasets are rich but complex, and disease-relevant information may be distributed across force curves, mechanical maps, adhesion patterns, topographical features, stiffness distributions, and spatial heterogeneity.

Despite this progress, most AI-enabled AFM biomarker studies remain at the proof-of-concept stage. The main limitations include small datasets, limited patient-derived material, restricted external validation, variation in AFM acquisition protocols, differences in sample preparation and data-processing pipelines, and the risk of overfitting or data leakage. These limitations are particularly important when many force curves, images, or maps are collected from the same cell, tissue, animal, or patient sample. Therefore, most AFM-derived nanomechanical biomarkers discussed in this review should currently be considered candidate biomarkers rather than clinically validated biomarkers.

Clinical translation will require larger and better-annotated datasets, standardized AFM acquisition and reporting protocols, independent validation cohorts, patient- or sample-level data splitting, transparent preprocessing, and reproducible AI/ML workflows. Explainable AI will also be essential to identify whether model predictions are driven by biologically meaningful features, such as stiffness heterogeneity, adhesion, viscoelasticity, roughness, or force-curve shape, rather than technical artifacts. In addition, integration with histopathology, optical imaging, molecular profiling, and clinical metadata will be needed to determine whether AFM-derived mechanical information provides added diagnostic, prognostic, or treatment-monitoring value.

Overall, AI/ML-assisted AFM has strong potential to support disease classification, treatment monitoring, and precision mechanobiology, especially in cancer and fibrotic disease. However, its successful translation will depend not only on algorithmic performance, but also on standardization, reproducibility, external validation, explainability, and clear demonstration of clinical utility.

Funding: This research was funded by the Research and Innovation Foundation (RIF) through the projects PDTenhance (EXCELLENCE/0524/0227) and MechanoNCoDe (VISION ERC/0524/0003), which is implemented under the programme of social cohesion "THALIA 2021-2027" co-funded by the European Union, through Research and Innovation Foundation.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The author declares no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AFM	Atomic force microscopy
AI	Artificial intelligence
AI/ML	Artificial intelligence and machine learning
Bio-AFM	Biological atomic force microscopy
CC BY	Creative Commons Attribution
CNN	Convolutional neural network
DMT	Derjaguin–Muller–Toporov
ECM	Extracellular matrix
EMT	Epithelial-to-mesenchymal transition
FAIR	Findable, accessible, interoperable, and reusable
HCC	Hepatocellular carcinoma
JKR	Johnson–Kendall–Roberts
LIME	Local interpretable model-agnostic explanations
ML	Machine learning
NMF	Nanomechanical fingerprint
NMFs	Nanomechanical fingerprints
PCA	Principal component analysis
PD-L1	Programmed death-ligand 1
PDT	Photodynamic therapy
SHAP	Shapley additive explanations
SHG	Second harmonic generation
SVM	Support vector machine
TME	Tumor microenvironment

References

1. Iskratsch, T.; Wolfenson, H.; Sheetz, M.P. Appreciating force and shape—The rise of mechanotransduction in cell biology. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 825–833. [CrossRef] [PubMed]
2. Romani, P.; Valcarcel-Jimenez, L.; Frezza, C.; Dupont, S. Crosstalk between mechanotransduction and metabolism. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 22–38. [PubMed]
3. Humphrey, J.D.; Dufresne, E.R.; Schwartz, M.A. Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 802–812. [CrossRef] [PubMed]
4. Uhler, C.; Shivashankar, G.V. Regulation of genome organization and gene expression by nuclear mechanotransduction. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 717–727. [CrossRef] [PubMed]
5. Lee, J.Y.; Chang, J.K.; Dominguez, A.A.; Lee, H.-p.; Nam, S.; Chang, J.; Varma, S.; Qi, L.S.; West, R.B.; Chaudhuri, O. YAP-independent mechanotransduction drives breast cancer progression. *Nat. Commun.* **2019**, *10*, 1848. [CrossRef] [PubMed]
6. Stylianou, A.; Kontomaris, S.V.; Alexandratou, E.; Grant, C. Atomic Force Microscopy on biological materials related to pathological conditions. *Scanning* **2019**, *2019*, 8452851. [CrossRef] [PubMed]
7. Stylianou, A.; Lekka, M.; Stylianopoulos, T. AFM assessing of nanomechanical fingerprints for cancer early diagnosis and classification: From single cell to tissue level. *Nanoscale* **2018**, *10*, 20930–20945. [CrossRef] [PubMed]
8. Stylianou, A.; Mpekris, F.; Voutouri, C.; Papoui, A.; Constantinidou, A.; Kitiris, E.; Kailides, M.; Stylianopoulos, T. Nanomechanical properties of solid tumors as treatment monitoring biomarkers. *Acta Biomater.* **2022**, *154*, 324–334. [CrossRef] [PubMed]
9. Stylianou, A.; Polemidiotou, K.; Alimisis, V.; Mpekris, F.; Voutouri, C.; Mari, M.; Filippidis, G.; Zachariades, A.; Kontomaris, S.-V.; Sotiriadis, P.P.; et al. Atomic Force Microscopy-Based Nanomechanical Signatures for Staging Classification and Drug Response in Pulmonary Fibrosis. *Small* **2025**, *21*, e04526. [CrossRef] [PubMed]
10. Garcia, R. Nanomechanical mapping of soft materials with the atomic force microscope: Methods, theory and applications. *Chem. Soc. Rev.* **2020**, *49*, 5850–5884. [CrossRef] [PubMed]
11. Morris, V.J.; Kirby, A.R.; Gunning, A.P. *Atomic Force Microscopy for Biologists*; Imperial College Press: London, UK, 2008.
12. Zhu, X.; Qin, R.; Qu, K.; Wang, Z.; Zhao, X.; Xu, W. Atomic force microscopy–based assessment of multimechanical cellular properties for classification of graded bladder cancer cells and cancer early diagnosis using machine learning analysis. *Acta Biomater.* **2023**, *158*, 358–373. [CrossRef] [PubMed]
13. Pacheco, L.R.L.; Ferreira, J.P.S.; Parente, M.P.L. Deep Learning Regressors of Surface Properties from Atomic Force Microscopy Nanoindentations. *Appl. Sci.* **2024**, *14*, 2376. [CrossRef]
14. Viljoen, A.; Mathelié-Guinlet, M.; Ray, A.; Strohmeyer, N.; Oh, Y.J.; Hinterdorfer, P.; Müller, D.J.; Alsteens, D.; Dufrêne, Y.F. Force spectroscopy of single cells using atomic force microscopy. *Nat. Rev. Methods Primers* **2021**, *1*, 63. [CrossRef]
15. Krieg, M.; Fläschner, G.; Alsteens, D.; Gaub, B.M.; Roos, W.H.; Wuite, G.J.L.; Gaub, H.E.; Gerber, C.; Dufrêne, Y.F.; Müller, D.J. Atomic force microscopy-based mechanobiology. *Nat. Rev. Phys.* **2019**, *1*, 41–57.
16. Nia, H.T.; Munn, L.L.; Jain, R.K. Probing the physical hallmarks of cancer. *Nat. Methods* **2025**, *22*, 1800–1818. [CrossRef] [PubMed]
17. O'Dowling, A.T.; Rodriguez, B.J.; Gallagher, T.K.; Thorpe, S.D. Machine learning and artificial intelligence: Enabling the clinical translation of atomic force microscopy-based biomarkers for cancer diagnosis. *Comput. Struct. Biotechnol. J.* **2024**, *24*, 661–671. [CrossRef] [PubMed]
18. Kontomaris, S.V.; Malamou, A.; Stylianou, A. The Young's Modulus as a Mechanical Biomarker in AFM Experiments: A Tool for Cancer Diagnosis and Treatment Monitoring. *Sensors* **2025**, *25*, 3510. [CrossRef] [PubMed]
19. Masud, N.R.; Rade, J.; Hasib, M.H.H.; Krishnamurthy, A.; Sarkar, A. Machine learning approaches for improving atomic force microscopy instrumentation and data analytics. *Front. Phys.* **2024**, *12*, 1347648. [CrossRef]
20. Bzdok, D.; Krzywinski, M.; Altman, N. Machine learning: A primer. *Nat. Methods* **2017**, *14*, 1119–1120. [CrossRef] [PubMed]
21. Sokolov, I. On machine learning analysis of atomic force microscopy images for image classification, sample surface recognition. *Phys. Chem. Chem. Phys.* **2024**, *26*, 11263–11270. [CrossRef] [PubMed]
22. Xiao, R.; Zhang, Y.; Li, M. Automated High-Throughput Atomic Force Microscopy Single-Cell Nanomechanical Assay Enabled by Deep Learning-Based Optical Image Recognition. *Nano Lett.* **2024**, *24*, 12323–12332. [CrossRef] [PubMed]
23. Thomas-Chemin, O.; Janel, S.; Boumehdi, Z.; Séverac, C.; Trevisiol, E.; Dague, E.; Duprés, V. Advancing High-Throughput Cellular Atomic Force Microscopy with Automation and Artificial Intelligence. *ACS Nano* **2025**, *19*, 5045–5062. [CrossRef] [PubMed]
24. Petrov, M.; Sokolov, I. Machine Learning Allows for Distinguishing Precancerous and Cancerous Human Epithelial Cervical Cells Using High-Resolution AFM Imaging of Adhesion Maps. *Cells* **2023**, *12*, 2536. [CrossRef] [PubMed]
25. Minelli, E.; Ciasca, G.; Sassun, T.E.; Antonelli, M.; Palmieri, V.; Papi, M.; Maulucci, G.; Santoro, A.; Giangaspero, F.; Delfini, R.; et al. A fully-automated neural network analysis of AFM force-distance curves for cancer tissue diagnosis. *Appl. Phys. Lett.* **2017**, *111*, 143701. [CrossRef]
26. Prasad, S.; Rankine, A.; Prasad, T.; Song, P.; Dokukin, M.E.; Makarova, N.; Backman, V.; Sokolov, I. Atomic Force Microscopy Detects the Difference in Cancer Cells of Different Neoplastic Aggressiveness via Machine Learning. *Adv. NanoBiomed Res.* **2021**, *1*, 2000116. [CrossRef]

27. Wu, H.; Zhang, L.; Zhao, B.; Yang, W.; Galluzzi, M. Deep learning strategy for small dataset from atomic force microscopy mechano-imaging on macrophages phenotypes. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1259979. [CrossRef] [PubMed]
28. Yang, X.; Yang, Y.; Zhang, Z.; Li, M. Deep Learning Image Recognition-Assisted Atomic Force Microscopy for Single-Cell Efficient Mechanics in Co-culture Environments. *Langmuir ACS J. Surf. Colloids* **2024**, *40*, 837–852.
29. Schillers, H.; Rianna, C.; Schäpe, J.; Luque, T.; Doschke, H.; Wälte, M.; Uriarte, J.J.; Campillo, N.; Michanetzis, G.P.A.; Bobrowska, J.; et al. Standardized Nanomechanical Atomic Force Microscopy Procedure (SNAP) for Measuring Soft and Biological Samples. *Sci. Rep.* **2017**, *7*, 5117. [CrossRef] [PubMed]
30. Mostaço-Guidolin, L.; Rosin, N.L.; Hackett, T.L. Imaging collagen in scar tissue: Developments in second harmonic generation microscopy for biomedical applications. *Int. J. Mol. Sci.* **2017**, *18*, 1772. [CrossRef] [PubMed]
31. Varoquaux, G.; Cheplygina, V. Machine learning for medical imaging: Methodological failures and recommendations for the future. *npj Digit. Med.* **2022**, *5*, 48. [CrossRef] [PubMed]
32. Bradshaw, T.J.; Huemann, Z.; Hu, J.; Rahmim, A. A Guide to Cross-Validation for Artificial Intelligence in Medical Imaging. *Radiol. Artif. Intell.* **2023**, *5*, e220232. [CrossRef] [PubMed]
33. Rosenblatt, M.; Tejavibulya, L.; Jiang, R.; Noble, S.; Scheinost, D. Data leakage inflates prediction performance in connectome-based machine learning models. *Nat. Commun.* **2024**, *15*, 1829. [CrossRef] [PubMed]
34. Klauschen, F.; Dippel, J.; Keyl, P.; Jurmeister, P.; Bockmayr, M.; Mock, A.; Buchstab, O.; Alber, M.; Ruff, L.; Montavon, G.; et al. Toward Explainable Artificial Intelligence for Precision Pathology. *Annu. Rev. Pathol.* **2024**, *19*, 541–570. [CrossRef] [PubMed]
35. Haase, K.; Pelling, A.E. Investigating cell mechanics with atomic force microscopy. *J. R. Soc. Interface* **2015**, *12*, 20140970. [CrossRef] [PubMed]
36. Calò, A.; Romin, Y.; Srouji, R.; Zambirinis, C.P.; Fan, N.; Santella, A.; Feng, E.; Fujisawa, S.; Turkekul, M.; Huang, S.; et al. Spatial mapping of the collagen distribution in human and mouse tissues by force volume atomic force microscopy. *Sci. Rep.* **2020**, *10*, 15664. [CrossRef] [PubMed]
37. Dufrêne, Y.F.; Ando, T.; Garcia, R.; Alsteens, D.; Martinez-Martin, D.; Engel, A.; Gerber, C.; Müller, D.J. Imaging modes of atomic force microscopy for application in molecular and cell biology. *Nat. Nanotechnol.* **2017**, *12*, 295. [CrossRef] [PubMed]
38. Cartagena-Rivera, A.X.; Wang, W.-H.; Geahlen, R.L.; Raman, A. Fast, multi-frequency, and quantitative nanomechanical mapping of live cells using the atomic force microscope. *Sci. Rep.* **2015**, *5*, 11692. [CrossRef] [PubMed]
39. Holuigue, H.; Lorenc, E.; Chighizola, M.; Schulte, C.; Varinelli, L.; Deraco, M.; Guaglio, M.; Gariboldi, M.; Podestà, A. Force Sensing on Cells and Tissues by Atomic Force Microscopy. *Sensors* **2022**, *22*, 2197. [CrossRef] [PubMed]
40. Deng, X.; Xiong, F.; Li, X.; Xiang, B.; Li, Z.; Wu, X.; Guo, C.; Li, X.; Li, Y.; Li, G.; et al. Application of atomic force microscopy in cancer research. *J. Nanobiotechnol.* **2018**, *16*, 102. [CrossRef] [PubMed]
41. Kontomaris, S.V.; Georgakopoulos, A.; Malamou, A.; Stylianou, A. The average Young's modulus as a physical quantity for describing the depth-dependent mechanical properties of cells. *Mech. Mater.* **2021**, *158*, 103846. [CrossRef]
42. Kontomaris, S.V.; Stylianou, A.; Nikita, K.; Malamou, A. A discussion regarding the application of the Hertz contact theory on biological samples in AFM nanoindentation experiments. *Micro Nanosyst.* **2021**, *13*, 42–48. [CrossRef]
43. Stylianos-Vasileios, K. The Hertz Model in AFM Nanoindentation Experiments: Applications in Biological Samples and Biomaterials. *Micro Nanosyst.* **2018**, *10*, 11–22. [CrossRef]
44. Kontomaris, S.V.; Malamou, A. Hertz model or Oliver & Pharr analysis? Tutorial regarding AFM nanoindentation experiments on biological samples. *Mater. Res. Express* **2020**, *7*, 033001. [CrossRef]
45. Polemidiotou, K.; Kulkarni, S.G.; Szydlak, R.; Lekka, M.; Radmacher, M.; Gkretsi, V.; Stylianopoulos, T.; Stylianou, A. Assessing sarcoma cell cytoskeleton remodeling in response to varying collagen concentration. *Int. J. Biol. Macromol.* **2024**, *282*, 136770. [CrossRef] [PubMed]
46. Stylianou, A.; Gkretsi, V.; Louca, M.; Zacharia, L.; Stylianopoulos, T. Collagen content and extracellular matrix cause cytoskeletal remodelling in pancreatic fibroblasts. *J. R. Soc. Interface* **2019**, *16*, 20190226. [CrossRef] [PubMed]
47. Stylianou, A.; Stylianopoulos, T. Atomic Force Microscopy Probing of Cancer Cells and Tumor Microenvironment Components. *BioNanoScience* **2016**, *6*, 33–46.
48. Lekka, M.; Laidler, P. Applicability of AFM in cancer detection. *Nat. Nanotechnol.* **2009**, *4*, 72. [CrossRef]
49. Lekka, M. Atomic force microscopy: A tip for diagnosing cancer. *Nat. Nanotechnol.* **2012**, *7*, 691–692. [PubMed]
50. Lekka, M.; Pogoda, K.; Gostek, J.; Klymenko, O.; Prauzner-Bechcicki, S.; Wiltowska-Zuber, J.; Jaczewska, J.; Lekki, J.; Stachura, Z. Cancer cell recognition–mechanical phenotype. *Micron* **2012**, *43*, 1259–1266. [CrossRef] [PubMed]
51. Stylianou, A.; Voutouri, C.; Mpekris, F.; Stylianopoulos, T. Pancreatic cancer presents distinct nanomechanical properties during progression. *Ann. Biomed. Eng.* **2023**, *51*, 1602–1615. [CrossRef] [PubMed]
52. Plodinec, M.; Loparic, M.; Monnier, C.A.; Obermann, E.C.; Zanetti-Dallenbach, R.; Oertle, P.; Hyotyla, J.T.; Aebi, U.; Bentires-Alj, M.; Lim, R.Y.; et al. The nanomechanical signature of breast cancer. *Nat. Nanotechnol.* **2012**, *7*, 757–765. [CrossRef] [PubMed]
53. Tian, M.; Li, Y.; Liu, W.; Jin, L.; Jiang, X.; Wang, X.; Ding, Z.; Peng, Y.; Zhou, J.; Fan, J.; et al. The nanomechanical signature of liver cancer tissues and its molecular origin. *Nanoscale* **2015**, *7*, 12998–13010. [CrossRef] [PubMed]

54. Júnior, C.; Narciso, M.; Marhuenda, E.; Almendros, I.; Farré, R.; Navajas, D.; Otero, J.; Gavara, N. Baseline Stiffness Modulates the Non-Linear Response to Stretch of the Extracellular Matrix in Pulmonary Fibrosis. *Int. J. Mol. Sci.* **2021**, *22*, 12928. [CrossRef] [PubMed]
55. Júnior, C.; Ulldemolins, A.; Narciso, M.; Almendros, I.; Farré, R.; Navajas, D.; López, J.; Eroles, M.; Rico, F.; Gavara, N. Multi-Step Extracellular Matrix Remodelling and Stiffening in the Development of Idiopathic Pulmonary Fibrosis. *Int. J. Mol. Sci.* **2023**, *24*, 1708. [CrossRef] [PubMed]
56. Ojha, S.; Pribyl, J.; Klimovic, S.; Hadraba, D.; Jirouskova, M.; Gregor, M. Measurement of Liver Stiffness using Atomic Force Microscopy Coupled with Polarization Microscopy. *J. Vis. Exp. JoVE* **2022**, e63974. [CrossRef] [PubMed]
57. Kontomaris, S.V.; Psychogios, I.; Stergiopoulos, M.; Malamou, A.; Stylianou, A. Quantitative criteria for the validity of the elastic half-space assumption in AFM nanoindentation. *Next Mater.* **2025**, *9*, 101180. [CrossRef]
58. Friedrichs, J.; Helenius, J.; Muller, D.J. Quantifying cellular adhesion to extracellular matrix components by single-cell force spectroscopy. *Nat. Protoc.* **2010**, *5*, 1353–1361. [CrossRef] [PubMed]
59. Le Cigne, A.; Chièze, L.; Beaussart, A.; El-Kirat-Chatel, S.; Dufrêne, Y.F.; Dedieu, S.; Schneider, C.; Martiny, L.; Devy, J.; Molinari, M. Analysis of the effect of LRP-1 silencing on the invasive potential of cancer cells by nanomechanical probing and adhesion force measurements using atomic force microscopy. *Nanoscale* **2016**, *8*, 7144–7154. [CrossRef] [PubMed]
60. Cooper, J.; Giancotti, F.G. Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance. *Cancer Cell* **2019**, *35*, 347–367. [CrossRef] [PubMed]
61. Smith, B.N.; Bhowmick, N.A. Role of EMT in Metastasis and Therapy Resistance. *J. Clin. Med.* **2016**, *5*, 17. [CrossRef] [PubMed]
62. Notbohm, J.; Poon, B.; Ravichandran, G. Analysis of nanoindentation of soft materials with an atomic force microscope. *J. Mater. Res.* **2011**, *27*, 229–237. [CrossRef]
63. Leite, F.L.; Bueno, C.C.; Da Róz, A.L.; Ziemath, E.C.; Oliveira, O.N. Theoretical Models for Surface Forces and Adhesion and Their Measurement Using Atomic Force Microscopy. *Int. J. Mol. Sci.* **2012**, *13*, 12773–12856. [CrossRef] [PubMed]
64. Efremov, Y.M.; Okajima, T.; Raman, A. Measuring viscoelasticity of soft biological samples using atomic force microscopy. *Soft Matter* **2020**, *16*, 64–81. [CrossRef] [PubMed]
65. Flormann, D.A.D.; Anton, C.; Pohland, M.O.; Bautz, Y.; Kaub, K.; Terriac, E.; Schäffer, T.E.; Rheinlaender, J.; Janshoff, A.; Ott, A.; et al. Oscillatory Microrheology, Creep Compliance and Stress Relaxation of Biological Cells Reveal Strong Correlations as Probed by Atomic Force Microscopy. *Front. Phys.* **2021**, *9*, 711860. [CrossRef]
66. Hadzipasic, M.; Zhang, S.; Huang, Z.; Passaro, R.; Sten, M.S.; Shankar, G.M.; Nia, H.T. Emergence of nanoscale viscoelasticity from single cancer cells to established tumors. *Biomaterials* **2024**, *305*, 122431. [CrossRef] [PubMed]
67. Nia, H.T.; Munn, L.L.; Jain, R.K. Physical traits of cancer. *Science* **2020**, *370*, eaaz0868. [CrossRef]
68. Guan, G.; Coates, D.E.; Sun, Q.; Cannon, R.D.; Mei, L. Atomic Force Microscopy for Revealing Oncological Nanomechanobiology and Thermodynamics. *ACS Nano* **2025**, *19*, 10862–10877. [CrossRef] [PubMed]
69. Petrov, M.; Sokolov, I. Identification of Geometrical Features of Cell Surface Responsible for Cancer Aggressiveness: Machine Learning Analysis of Atomic Force Microscopy Images of Human Colorectal Epithelial Cells. *Biomedicines* **2023**, *11*, 191. [CrossRef] [PubMed]
70. Haydak, J.; Azeloglu, E.U. Automated atomic force microscopy analysis using convolutional and recurrent neural networks. *Biophys. J.* **2025**, *124*, 1891–1901. [CrossRef] [PubMed]
71. Zeng, Y.; Liu, X.; Wang, Z.; Gao, W.; Li, L.; Zhang, S. Detection and classification of hepatocytes and hepatoma cells using atomic force microscopy and machine learning algorithms. *Microsc. Res. Tech.* **2023**, *86*, 1047–1056. [CrossRef] [PubMed]
72. Dokukin, M.; Dokukina, I. Application of ensemble machine learning methods to multidimensional AFM data sets. *Procedia Comput. Sci.* **2020**, *169*, 763–766. [CrossRef]
73. Li, K.; Yao, S.; Zhang, Z.; Cao, B.; Wilson, C.M.; Kalos, D.; Kuan, P.F.; Zhu, R.; Wang, X. Efficient gradient boosting for prognostic biomarker discovery. *Bioinformatics* **2022**, *38*, 1631–1638. [CrossRef] [PubMed]
74. Carracedo-Cosme, J.; Romero-Muñiz, C.; Pérez, R. A Deep Learning Approach for Molecular Classification Based on AFM Images. *Nanomaterials* **2021**, *11*, 1658. [CrossRef] [PubMed]
75. Ansardamavandi, A.; Tafazzoli-Shadpour, M.; Omidvar, R.; Jahanzad, I. Quantification of effects of cancer on elastic properties of breast tissue by Atomic Force Microscopy. *J. Mech. Behav. Biomed. Mater.* **2016**, *60*, 234–242. [CrossRef] [PubMed]
76. Ansardamavandi, A.; Tafazzoli-Shadpour, M.; Omidvar, R.; Nili, F. An AFM-Based Nanomechanical Study of Ovarian Tissues with Pathological Conditions. *Int. J. Nanomed.* **2020**, *15*, 4333–4350. [CrossRef] [PubMed]
77. Weber, A.; Vivanco, M.d.; Toca-Herrera, J.L. Application of self-organizing maps to AFM-based viscoelastic characterization of breast cancer cell mechanics. *Sci. Rep.* **2023**, *13*, 3087. [CrossRef] [PubMed]
78. Budich, C.; West, J.; Lampen, P.; Deckert, V. Force microscopy analysis using chemometric tools. *Anal. Bioanal. Chem.* **2008**, *390*, 1253–1260. [CrossRef] [PubMed]
79. Paruchuri, A.; Wang, Y.; Gu, X.; Jayaraman, A. Machine learning for analyzing atomic force microscopy (AFM) images generated from polymer blends. *Digit. Discov.* **2024**, *3*, 2533–2550. [CrossRef]

80. Sotres, J.; Boyd, H.; Gonzalez-Martinez, J.F. Locating critical events in AFM force measurements by means of one-dimensional convolutional neural networks. *Sci. Rep.* **2022**, *12*, 12995. [CrossRef] [PubMed]
81. Corrias, M.; Papa, L.; Sokolović, I.; Birschitzky, V.; Gorfer, A.; Setvin, M.; Schmid, M.; Diebold, U.; Reticcioli, M.; Franchini, C. Automated real-space lattice extraction for atomic force microscopy images. *Mach. Learn. Sci. Technol.* **2023**, *4*, 015015. [CrossRef]
82. Topol, E.J. High-performance medicine: The convergence of human and artificial intelligence. *Nat. Med.* **2019**, *25*, 44–56. [CrossRef] [PubMed]
83. Lundberg, S.M.; Lee, S.-I. A unified approach to interpreting model predictions. In *Proceedings of the 31st International Conference on Neural Information Processing Systems, Long Beach, California, USA*; Curran Associates Inc.: Nice, France, 2017; pp. 4768–4777.
84. Ribeiro, M.T.; Singh, S.; Guestrin, C. "Why Should I Trust You?": Explaining the Predictions of Any Classifier. In *Proceedings of the 22nd ACM SIGKDD International Conference on Knowledge Discovery and Data Mining, San Francisco, California, USA*; Association for Computing Machinery: New York, NY, USA, 2016; pp. 1135–1144.
85. Selvaraju, R.R.; Cogswell, M.; Das, A.; Vedantam, R.; Parikh, D.; Batra, D. Grad-CAM: Visual Explanations from Deep Networks via Gradient-Based Localization. In Proceedings of the 2017 IEEE International Conference on Computer Vision (ICCV), Venice, Italy, 22–29 October 2017; pp. 618–626.
86. Bach, S.; Binder, A.; Montavon, G.; Klauschen, F.; Müller, K.R.; Samek, W. On Pixel-Wise Explanations for Non-Linear Classifier Decisions by Layer-Wise Relevance Propagation. *PLoS ONE* **2015**, *10*, e0130140. [CrossRef] [PubMed]
87. He, K.; Gan, C.; Li, Z.; Rekik, I.; Yin, Z.; Ji, W.; Gao, Y.; Wang, Q.; Zhang, J.; Shen, D. Transformers in medical image analysis. *Intell. Med.* **2023**, *3*, 59–78. [CrossRef]
88. Huang, S.-C.; Pareek, A.; Jensen, M.; Lungren, M.P.; Yeung, S.; Chaudhari, A.S. Self-supervised learning for medical image classification: A systematic review and implementation guidelines. *npj Digit. Med.* **2023**, *6*, 74. [CrossRef] [PubMed]
89. Moor, M.; Banerjee, O.; Abad, Z.S.H.; Krumholz, H.M.; Leskovec, J.; Topol, E.J.; Rajpurkar, P. Foundation models for generalist medical artificial intelligence. *Nature* **2023**, *616*, 259–265. [CrossRef] [PubMed]
90. Varoquaux, G. Cross-validation failure: Small sample sizes lead to large error bars. *NeuroImage* **2018**, *180*, 68–77. [CrossRef] [PubMed]
91. Kapoor, S.; Narayanan, A. Leakage and the reproducibility crisis in machine-learning-based science. *Patterns* **2023**, *4*, 100804. [CrossRef] [PubMed]
92. Tampu, I.E.; Eklund, A.; Haj-Hosseini, N. Inflation of test accuracy due to data leakage in deep learning-based classification of OCT images. *Sci. Data* **2022**, *9*, 580. [CrossRef] [PubMed]
93. Ciasca, G.; Sassun, T.E.; Minelli, E.; Antonelli, M.; Papi, M.; Santoro, A.; Giangaspero, F.; Delfini, R.; De Spirito, M. Nano-mechanical signature of brain tumours. *Nanoscale* **2016**, *8*, 19629–19643. [CrossRef] [PubMed]
94. Cieśluk, M.; Pogoda, K.; Deptuła, P.; Werel, P.; Kułakowska, A.; Kochanowicz, J.; Mariak, Z.; Łysoń, T.; Reszeć, J.; Bucki, R. Nanomechanics and Histopathology as Diagnostic Tools to Characterize Freshly Removed Human Brain Tumors. *Int. J. Nanomed.* **2020**, *15*, 7509–7521. [CrossRef] [PubMed]
95. Cross, S.E.; Jin, Y.S.; Rao, J.; Gimzewski, J.K. Nanomechanical analysis of cells from cancer patients. *Nat. Nanotechnol.* **2007**, *2*, 780–783. [CrossRef] [PubMed]
96. Bae, Y.H.; Liu, S.L.; Byfield, F.J.; Janmey, P.A.; Assoian, R.K. Measuring the Stiffness of Ex Vivo Mouse Aortas Using Atomic Force Microscopy. *J. Vis. Exp. JoVE* **2016**, e54630. [CrossRef] [PubMed]
97. Sicard, D.; Fredenburgh, L.E.; Tschumperlin, D.J. Measured pulmonary arterial tissue stiffness is highly sensitive to AFM indenter dimensions. *J. Mech. Behav. Biomed. Mater.* **2017**, *74*, 118–127. [CrossRef] [PubMed]
98. Aguilar, V.M.; Paul, A.; Lazarko, D.; Levitan, I. Paradigms of endothelial stiffening in cardiovascular disease and vascular aging. *Front. Physiol.* **2023**, *13*, 1081119. [CrossRef] [PubMed]
99. Darling, E.M.; Wilusz, R.E.; Bolognesi, M.P.; Zauscher, S.; Guilak, F. Spatial mapping of the biomechanical properties of the pericellular matrix of articular cartilage measured in situ via atomic force microscopy. *Biophys. J.* **2010**, *98*, 2848–2856. [CrossRef] [PubMed]
100. Danalache, M.; Kleinert, R.; Schneider, J.; Erler, A.L.; Schwitalle, M.; Riester, R.; Traub, F.; Hofmann, U.K. Changes in stiffness and biochemical composition of the pericellular matrix as a function of spatial chondrocyte organisation in osteoarthritic cartilage. *Osteoarthr. Cartil.* **2019**, *27*, 823–832. [CrossRef] [PubMed]
101. Puttini, S.; Lekka, M.; Dorchies, O.M.; Saugy, D.; Incitti, T.; Ruegg, U.T.; Bozzoni, I.; Kulik, A.J.; Mermod, N. Gene-mediated restoration of normal myofiber elasticity in dystrophic muscles. *Mol. Ther.* **2009**, *17*, 19–25. [CrossRef] [PubMed]
102. Watanabe-Nakayama, T.; Ono, K.; Itami, M.; Takahashi, R.; Teplow, D.B.; Yamada, M. High-speed atomic force microscopy reveals structural dynamics of amyloid β_{1-42} aggregates. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 5835–5840. [CrossRef] [PubMed]
103. do Nascimento Amorim, M.d.S.; Silva França, Á.R.; Santos-Oliveira, R.; Rodrigues Sanches, J.; Marinho Melo, T.; Araújo Serra Pinto, B.; Barbosa, L.R.S.; Alencar, L.M.R. Atomic Force Microscopy Applied to the Study of Tauopathies. *ACS Chem. Neurosci.* **2024**, *15*, 699–715. [CrossRef] [PubMed]

104. Huang, Y.; Kyriakides, T.R. The role of extracellular matrix in the pathophysiology of diabetic wounds. *Matrix Biol. Plus* **2020**, *6-7*, 100037. [CrossRef] [PubMed]
105. Miler, I.; Rabasovic, M.D.; Askrabic, S.; Stylianou, A.; Korac, B.; Korac, A. Short-Term l-arginine Treatment Mitigates Early Damage of Dermal Collagen Induced by Diabetes. *Bioengineering* **2024**, *11*, 407. [CrossRef] [PubMed]
106. Loyola-Leyva, A.; Loyola-Rodríguez, J.P.; Terán-Figueroa, Y.; Camacho-Lopez, S.; González, F.J.; Barquera, S. Application of atomic force microscopy to assess erythrocytes morphology in early stages of diabetes. A pilot study. *Micron* **2021**, *141*, 102982. [CrossRef] [PubMed]
107. Wenderott, J.K.; Flesher, C.G.; Baker, N.A.; Neeley, C.K.; Varban, O.A.; Lumeng, C.N.; Muhammad, L.N.; Yeh, C.; Green, P.F.; O'Rourke, R.W. Elucidating nanoscale mechanical properties of diabetic human adipose tissue using atomic force microscopy. *Sci. Rep.* **2020**, *10*, 20423. [CrossRef] [PubMed]
108. Mpekris, F.; Panagi, M.; Charalambous, A.; Voutouri, C.; Stylianopoulos, T. Modulating cancer mechanopathology to restore vascular function and enhance immunotherapy. *Cell Rep. Med.* **2024**, *5*, 101626. [CrossRef] [PubMed]
109. Matias, Z.; Lopes, C.S.; Santos, N.C.; Carvalho, F.A. Nanotechnology meets medicine: Applications of atomic force microscopy in disease. *Biophys. Rev.* **2025**, *17*, 359–384. [CrossRef] [PubMed]
110. Gavriil, V.; Ferraro, A.; Cefalas, A.-C.; Kollia, Z.; Pepe, F.; Malapelle, U.; De Luca, C.; Troncone, G.; Sarantopoulou, E. Nanoscale Prognosis of Colorectal Cancer Metastasis from AFM Image Processing of Histological Sections. *Cancers* **2023**, *15*, 1220. [CrossRef] [PubMed]
111. Panagi, M.; Mpekris, F.; Chen, P.; Voutouri, C.; Nakagawa, Y.; Martin, J.D.; Hiroi, T.; Hashimoto, H.; Demetriou, P.; Pierides, C.; et al. Polymeric micelles effectively reprogram the tumor microenvironment to potentiate nano-immunotherapy in mouse breast cancer models. *Nat. Commun.* **2022**, *13*, 7165. [CrossRef] [PubMed]
112. Voutouri, C.; Panagi, M.; Mpekris, F.; Stylianou, A.; Michael, C.; Averkiou, M.A.; Martin, J.D.; Stylianopoulos, T. Endothelin Inhibition Potentiates Cancer Immunotherapy Revealing Mechanical Biomarkers Predictive of Response. *Adv. Ther.* **2021**, *4*, 2000289. [CrossRef]
113. Panagi, M.; Mpekris, F.; Voutouri, C.; Hadjigeorgiou, A.G.; Symeonidou, C.; Porfyriou, E.; Michael, C.; Stylianou, A.; Martin, J.D.; Cabral, H.; et al. Stabilizing Tumor-Resident Mast Cells Restores T-Cell Infiltration and Sensitizes Sarcomas to PD-L1 Inhibition. *Clin. Cancer Res.* **2024**, *30*, 2582–2597. [CrossRef] [PubMed]
114. Torna, S.; Gkretsi, V.; Stylianou, A. Photodynamic Therapy and Tumor Microenvironment-Targeting Strategies: A Novel Synergy at the Frontier of Cancer Treatment. *Int. J. Mol. Sci.* **2025**, *26*, 8588. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.