European Partnership on Metrology
Call 2025 – Health, Integrated European Metrology, Regulation and Research Potential

Selected Research Topic number: **SRT-h03**
Version: 1.0

Title: Metrology for reliable biomechanical phenotyping aimed at cancer tissue diagnosis

Abstract

Reliable clinical application of nanoscale biomechanical phenotyping for early cancer detection and diagnosis requires addressing critical metrological challenges inherent to the methods, along with ensuring fast, reliable data analysis. Tacking these challenges requires the development of soft reference materials with tissue-like properties, establishing traceable metrology for atomic force microscope (AFM) cantilever stiffness calibration and tip area characterisation, as well as standardising bio-AFM procedures for viscoelastic tissue measurements with support of machine learning implementation.

Keywords

Biomechanical phenotyping, early cancer detection, nanomechanical metrology, soft reference material, atomic force microscopy (AFM), cancer tissue mechanics, early-stage diagnosis, viscoelastic properties.

Background to the Metrological Challenges

Over the past two decades, extensive experimental studies have proven that the biomechanical properties of cells and tissues vary as a function of their tumorigenicity, metastatic potential, and health state. Among various mechanobiological techniques such as micropipette aspiration, optical tweezers, and microfluidic devices, atomic force microscopy (AFM) stands out due to its nanomechanical measurement capabilities at both the cellular and tissue levels, offering exceptional spatial resolution. Compared to non-invasive techniques like Brillouin microscopy, AFM-based biomechanical phenotyping provides higher resolution and reliability for measuring tissue mechanical properties. Bio-AFM studies have shown significant changes in biomechanical properties with disease onset, particularly cancer. As a result, nanomechanical AFMs have emerged as a powerful tool for understanding life processes, revealing how changes in molecular, architectural, and behavioural properties relate to disease progression, and correlating tissue mechanics with cancer progression. However, biomechanical phenotyping with bio-AFM has not been adopted clinically as yet, mainly because the following critical issues related to instrumentation, characterisation, and data evaluation and analysis for cancer tissue diagnostics need to be reliably addressed.

Reference materials (RM) are typically used for instrument and indenter tip calibration (e.g. ISO 14577-2 for hard materials and nano-indenters). To date, typical RM for mechanical measurements have mechanical properties ranging from 70 GPa (e.g. Quartz) to a few GPa (e.g. Polycarbonate). However, the typical mechanical properties of human tissues vary with elastic moduli down to 100 Pa. There are no current candidate RMs in the very compliant modulus regime (100 Pa to ~1 MPa) needed for characterisation and calibration of bio-AFMs used in nanomechanical measurements of biomaterials.

Reliable AFM nanomechanical measurements require well-developed and well-calibrated instruments, particularly for determining 1) AFM cantilever stiffness with uncertainties down to 5 % and 2) tip contact area uncertainty below 10 %. Biomechanical AFM measurements are often performed in physiological buffers, making stiffness calibration in liquid essential. AFM indentation uncertainty is highly influenced by probe tip geometry. Studies show sharp tips yield stiffness values 2–3 times greater than spherical probes, emphasising the need for accurate tip area function modelling. Non-destructive characterisation of sharp AFM probe tips using soft reference materials offers flexibility, cost-effectiveness, and convenience.

EURAMET MSU,
Email: part.msu@euramet.org
Metpart.eu

European Partnership Co-funded by the European Union
METROLOGY PARTNERSHIP EURAMET

Tip area function characterisation for deep indentations (> 200 nm) lacks a standardised model. Common probe shapes—pyramidal, flat punch, and spherical—are often approximated by cones, paraboloids, or pyramids, leading to systematic errors. Generalised tip area functions with soft reference material-based method for traceable AFM tip area characterisation at deep indentations are urgently needed.

Both the elastic and viscoelastic properties of cells and tissues are validated biomarkers for cancer diagnosis. Standardised measurement procedures with open-source data interpretation models and software for both quasi-static and dynamic nanomechanical analysis are needed to reliably obtain the viscous biomechanical phenotypes of soft tissues. A key challenge is the quantitative conversion between rheological results (e.g., creep compliance, relaxation modulus) and complex moduli from dynamic AFM measurements. Interlaboratory multi-scale comparison measurements on soft reference materials are needed to validate the developed procedures, models and software for bio-AFMs, nanoindentation instruments, and microelectromechanical system (MEMS)-based scanning probe microscope (MEMS-SPM).

Despite growing awareness and EU-funded initiatives, no validated bio-AFM measurement procedures exist for viscoelastic tissue properties.

Bio-AFM biomechanical phenotyping could enhance histopathological analysis, but clinical adoption is hindered by complex, time-intensive data analysis requiring specialised expertise and personnel. A machine learning (ML) driven data analysis toolkit is urgently needed to automate i) preprocessing, qualification and sorting of bio-AFM datasets; ii) predictive selection of optimal contact mechanics models for tissue analysis; iii) evaluation of elastic and viscoelastic properties; iv) high-accuracy discrimination of malignant versus healthy tissues. To develop and validate this toolkit, interlaboratory comparisons on clinically relevant tissues are essential. Proposals should focus on preparing and distributing solid tumour samples, including decellularised colon tissue from colorectal cancer surgeries, for multi-scale bio-AFM measurements following the procedure developed. A feasibility study will assess ML-assisted AFM-based cancer diagnosis for precision surgery.

Currently, artificial intelligence (AI) has been applied to accelerate cancer tissue classification, but its effectiveness is limited by the lack of phenotypically rich AFM nanomechanical datasets. Comprehensive clinical tumour datasets are required for robust ML training. Additionally, critical patient data (e.g., age, sex, genetic mutations) remain underutilised in AI-driven diagnostics.

In summary, advancing AFM-based biomechanical phenotyping for early-stage clinical cancer detection requires developing metrological methods to address key instrumentation challenges, including reference materials, AFM probe stiffness calibration and tip contact area characterisation, and standardised measurement procedures. Furthermore, ML-powered AFM data analysis is essential for operator-independent, high-throughput cancer tissue diagnosis, ensuring enhanced sensitivity and reliability.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The proposal shall focus on providing the metrological framework, technical capability, and scientific knowledge to enable reliable and traceable characterisation of biomechanical properties of cells and tissues of clinical relevance for early detection and diagnosis of diseases, such as cancer.

The specific objectives are:

1. To develop soft reference materials (sRM) with tuneable elastic and viscoelastic properties, matching the elastic moduli of human tissue. This includes quantitative characterisation of quasi-static and dynamic mechanical properties for viscoelastic reference materials with moduli ranging from 100 Pa to 1 MPa to, ensuring the expanded measurement uncertainty within 10 % as well as assessing aging effects on the sRM properties under typical biological liquid media conditions mimicking at least 6 months of use and/or storage.
2. To develop calibration and characterisation methods to ensure the reliability and metrological traceability of nanoscale biomechanical measuring instruments. This includes i) traceable stiffness calibration methods for bio-atomic force microscopy (bio-AFM) cantilevers in both air and fluid with an expanded measurement uncertainty down to 5 %, and ii) traceable methodologies for quantitatively characterising the tip area function of sharp, flat punch and spherical AFM tips using the soft reference materials (sRM) developed in Objective 1. The focus will be on deep indentations beyond 200 nm to a few microns, commonly used in biological measurements.

3. To develop traceable quasi-static and dynamic nanomechanical measurement procedures for viscous biological tissues using atomic force microscopy (AFM) nanoindentation and micro-rheology at indentation depths from 200 nm to a few microns. This includes data interpretation models, algorithms, and open-source software for extracting the mechanical properties of viscous biomaterials, as well as creating a conversion algorithm to translate rheological data into complex frequency-dependent indentation moduli and vice versa for reference materials. A multi-scale comparison measurement campaign will be conducted to validate the models, software, and uncertainty budgets using the developed soft reference materials (sRM) and traceably calibrated bio-atomic force microscopy (bio-AFMs), nanoindentation instruments, and MEMS-based scanning probe microscopes (MEMS-SPM).
4. To develop and validate an open-access, machine-learning (ML)-driven bio-atomic force microscopy (bio-AFM) data analysis framework for high-sensitivity and high-accuracy clinical cancer tissue diagnosis. This includes i) the development of an open-access ML toolkit for automated bio-AFM data processing (data qualification and sorting, model selection, tissue property extraction, cancer differentiation), ii) the realisation of a interlaboratory comparison measurements to validate measurement procedures on clinically relevant cancer specimens as well as to assemble a multicentre dataset to train, validate, and benchmark the ML algorithm's diagnostic performance and iii) a feasibility study to evaluate the clinical utility of ML-assisted biomechanical diagnostics in informing precision surgical decision-making, with a focus on intraoperative tissue characterisation.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers), standards developing organisations (ISO/TC 164, ISO/TC 201, ISO/TC 229), and end users (e.g. clinical stakeholders, manufacturers of medical and healthcare products).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners, medical (academic) hospitals and industry is strongly recommended, both prior to and during methodology development. Where relevant, proposals are encouraged to build on, or seek collaboration with, existing projects and develop synergies with other relevant European, national or regional initiatives and funding programmes. In particular, links are encouraged with (i) the projects funded under earlier relevant topics of the Horizon Europe programme; or (ii) other relevant European Partnerships.

Proposers should establish the current state of the art and explain how their proposed project goes beyond this. In particular, proposers should outline the achievements of the EMRP project NEW05 MechProNo and EMRP project IND05 MeProVisc and how their proposal will build on those.

Proposers should note that the programme funds the activity of researchers to develop the capability, not the required infrastructure and capital equipment, which must be provided from other sources.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 2.1 M€ and has defined an upper limit of 2.6 M€ for this proposal.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Any industrial beneficiaries that will receive significant benefit from the results of the proposed project are expected to be beneficiaries without receiving funding or associated partners.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the 'end user' community, describing how the project participants will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the "end user" community (e.g. letters of support) is also encouraged.

You should detail how your proposal's results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Facilitate improved industrial capability, or improved quality of life for European citizens in terms of personal health, protection of the environment and the climate, or energy security,
- Transfer knowledge to the medical, healthcare and nanobiomechanical measurement sectors.

You should detail other impacts of your proposed JRP as specified in the document “Guide 4: Writing Joint Research Projects (JRPs)”

You should also detail how your approach to realising the objectives will further the aim of the Metrology Partnership to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work.

Timescale

The project should be of up to 3 years duration.