European Partnership on Metrology
Call 2022 – Digital Transformation, Health, Integrated European Metrology, Normative and Research Potential

Selected Research Topic number: **SRT-h08**
Version: 1.0

Title: Metrology for reliable characterisation of bioelectromechanical properties of tissue down to the nanoscale

Abstract

Existing methods for the measurement of the mechanical properties of single cells and tissues have shown that their bioelectromechanical properties change significantly with the onset of diseases such as cancer. This has clear implications for early disease detection and diagnosis and for the development of treatment plans. However, cells and tissues have very low elastic moduli which means that nanoscale responses need to be reliably measured. In addition, these existing methods are also unsuitable for use with viscous biological materials. Therefore, innovative reference materials with elastic moduli down to 100 Pa, standardised methods for dynamic nanomechanical measurements and device calibration guidelines need to be developed.

Keywords

Atomic force microscopy, biological properties, biomolecular interactions, biomolecule/cell detection, calibration methods, cancerous cells, characterisation, early-stage diagnosis, interlaboratory comparison, micro-rheology, nanoindentation, nanomechanical metrology, reference materials, tumour/soft tissue mechanics, ultrasoft AFM cantilevers

Background to the Metrological Challenges

Contact-based nanomechanical and electrical measurement approaches, including atomic force microscopy (AFM), have shown that the bioelectromechanical properties of cells and tissues change significantly with the onset of diseases such as cancer. The reliable characterisation of these properties down to the nanoscale could provide a better understanding of cancer initiation, progression, dissemination and metastasis.

The Young's modulus of a reference material provides the standard method for determining the instrument's key parameters for use in nanomaterial testing and for the evaluation of the overall performance of material measurement devices. The mechanical properties of cells and tissues vary widely, from cell membranes with moduli down to 100 Pa to bones with moduli up to 20 GPa. Therefore, typical reference materials for nanomechanical measurements have mechanical properties ranging from 70 GPa to ~ 10 MPa. Reference materials including polydimethylsiloxane and polyvinyl alcohol hydrogels, which have low viscosity and an elastic modulus ranging from a few kPa to MPa, are frequently used in mechanobiology. However, biological cells and tissues are usually highly viscous. Therefore, ultra-compliant reference materials with tuneable elastic and viscoelastic properties, which achieve similar elastic moduli to human tissue, need to be developed. The quasi-static and dynamic mechanical properties of these materials (elastic moduli: 10 MPa - 100 Pa, uncertainties of 10 %) also needs to be traceably characterised. In addition, the aging of these materials in liquid media needs to be measured and calibration substrates need to be developed for in-liquid measurement of the piezoelectric (0.01 pC/N - > 1000 pC/N) and dielectric (1 - 100) properties of these materials at the nanoscale (50 nm spatial resolution).

Contact and non-contact-based measurement methods are well established for the quantitative calibration of the bending stiffness of conventional AFM cantilevers in the 100 N/m to 0.1 N/m range. The thermal noise method can usually achieve uncertainties of ~ 15 %. Further research is needed to develop traceable stiffness calibration methods for ultrasoft AFM cantilevers (k_c down to 0.005 N/m, 5 % uncertainty) and calibration methods for the AFM tip contact area (10 % expanded uncertainty). For large indentation depths, which are unavoidable when measuring very soft samples, micro-machined silicon double springs could be used for the calibration of large indentation forces and depths. However, commercial products do not work for indentation

forces *F* << 100 µN and indentation depths h << 25 µm. Therefore, traceable probing force calibration methods (1 µN - 100 pN, 5 % expanded uncertainty) and force reference tools for *in-situ* stiffness (down to 0.01 N/m), deflection (up to 2 µm) and force calibration (10 nN - 1 µN) need to be developed. In addition, electrical calibration methods, including quantification of the piezoelectric and dielectric effects (< 10 % expanded uncertainty), also need to be developed.

The AFM indentation of soft biomaterials with sharp tips at high speed is essentially viscoelastic-viscoplastic material testing. Further research in this area will require traceable quasi-static and dynamic nanomechanical measurement procedures, such as AFM nanoindentation and micro-rheology, to be further developed for use with viscous biological tissues. Standardised data evaluation, including the well-known Oliver-Pharr model, can be used to evaluate the mechanical properties of elastoplastic materials. However, these approaches need to be further developed, including a thorough uncertainty analysis, for use with biomaterials. This should include modelling the validated contact mechanics and verifying this experimentally with viscoelastic and viscoplastic materials. In addition, micro-rheological data should be converted into complex moduli of indentation (frequency-dependent) and *vice versa* for reference materials. Stiffness-related prognostic/disease markers will also need to be discovered. The AFM measurement of cells suffers from low throughput due to their limited loading/unloading rates. In addition, measurements on tissues are even more challenging because of their complexity and heterogeneity. Therefore, high throughput nanomechanical measurement systems need to be validated at 1000 cells/h, demonstrating automated single-cell recognition, data analysis and interoperable file formats.

There are no validated methods to measure the nanomechanical properties of cells and tissues at the nano and micro scale. Consequently, there have been no interlaboratory comparisons. In addition, the metrology infrastructure needs to be developed. Therefore, the proposed new nanomechanical measurement procedures will need to be compared by measuring the properties of novel reference materials (with a target deviation of ~ 10 %). The recent ISO 21222 standard does not cover the essential mechanical properties of biological materials including the hardness and viscosity of cells and tissues. Therefore, standardised measurement approaches and data evaluation need to be developed for cells and tissues.

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 JRP shall focus on the traceable measurement and characterisation of the bioelectromechanical properties of cells and tissues, of clinical relevance, down to the nanoscale for the early detection and diagnosis of diseases like cancer.

The specific objectives are

1. To develop ultra-compliant reference materials with tuneable elastic and viscoelastic properties that achieve similar elastic moduli to human tissue. This should include traceable characterisation of the quasi-static and dynamic mechanical properties of these materials (elastic moduli: 10 MPa - 100 Pa, uncertainties of 10 %). To measure the aging of these materials in liquid media and to develop calibration substrates for in-liquid measurement of the piezoelectric (0.01 pC/N - > 1000 pC/N) and dielectric (1 - 100) properties of these materials at the nanoscale (50 nm spatial resolution).
2. To develop traceable stiffness calibration methods for ultrasoft AFM cantilevers (down to 0.005 N/m, 5 % uncertainty) and calibration methods for the AFM tip contact area (10 % expanded uncertainty). To develop traceable probing force calibration methods (1 µN - 100 pN, 5 % expanded uncertainty) and force reference tools for *in-situ* stiffness (down to 0.01 N/m), deflection (up to 2 µm) and force calibration (10 nN - 1 µN). In addition, to develop electrical calibration methods, including quantification of piezoelectric and dielectric effects (< 10 % expanded uncertainty).
3. To develop traceable quasi-static and dynamic nanomechanical measurement procedures for viscous biological tissues using AFM nanoindentation and micro-rheology. To model the validated contact mechanics and verify this experimentally with viscoelastic and viscoplastic materials. The micro-rheological results should be converted into complex moduli of indentation (frequency-dependent) and *vice versa* for reference materials. In addition, to discover stiffness-related prognostic/disease markers and validate high throughput nanomechanical measurement systems at 1000 cells/h, demonstrating automated single-cell recognition, data analysis and interoperable file formats.
4. To compare the nanomechanical measurement procedures, developed in objective 3, by measuring the properties of the reference materials developed in objective 1: results should deviate by ~ 10 %.

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 TC164, ISO TC201, ISO TC229, CEN TC352), 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.

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 projects IND05 MeProVisc and NEW05 MechProNo and how their proposal will build on those.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.9 M€ and has defined an upper limit of 2.6 M€ for this project.

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 partners 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 JRP 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,
- Transfer knowledge to the healthcare sector.

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 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.

Time-scale

The project should be of up to 3 years duration.