European Partnership on Metrology
Call 2024 –Digital Transformation, Green Deal, Normative and Research Potential

Selected Research Topic number: **SRT-n03**
Version: 1.0

Title: Standardisation of AFM nanomechanical measurements of biological and soft materials

Abstract

Reliable nanomechanical measurement of soft biomaterial and biological materials including living cells and tissues at the nanoscale requires standardisation due to their increase applications in healthcare, engineering biology (e.g. biomaterial manufacturing) and food technology and formulation. Atomic force microscopy (AFM) is the key method used for the measurement of biomechanical properties but there are no validated methods to measure the biomechanical properties at the nano and micro scale. In this proposal, soft candidate reference materials with elastic moduli ranging from 100 MPa down to 1 kPa will be established. In addition, key metrological developments in tip area function and force calibration will be progressed via standardised good practice guides, data analysis models, software and international ISO standards. The proposal outcomes will promote the standardisation of nanomechanical measurement of biological and soft materials, and contribute to reliable applications of bio-AFM, including early cancer diagnosis.

Keywords

Reference materials, biological materials, biomaterials, atomic force microscopy (AFM), Bio-AFM, nanomechanical measurement, nanoindentation, elastic modulus, nanohardness, Sader method, standardisation, nanoscale.

Background to the Metrological Challenges

Atomic force microscopy (AFM) is an increasingly important tool in biological and biomedical fields to image and map mechanical properties such as viscoelasticity, hardness and complex elastic modulus with very high nanoscale resolution and high force sensitivity down to pico-Newtons. AFM offers significant advantages over other microscopies since it is a non-destructive and precise 3D imaging method operating in samples' native environment. It can measure a wide size range of biological samples, from tiny DNA strands to bacteria and live cell sizes. Biomechanical properties of cells and tissues change significantly with the onset of certain classes of diseases, and nanomechanical measurements using bio-atomic force microscopy (bio-AFM) can help on the diagnosis, progression, and dissemination of cancer. It also has applications in food technology and formulation, health care and consumer healthcare and engineering biology (e. g. biomaterials). The biomaterials market is expected to experience strong demand from producers and users of high-quality control of artificial materials requiring AFM nanomechanical measurements.

Nevertheless, metrological developments are behind industrial and medical quality requirements in the rapid evolving use of AFM. For example, the available reference materials (RMs) can be used for instrument and indenter tip calibration, but they only reach measurements of mechanical properties ranging from 70 GPa (e.g. Quartz) to a few GPa (e.g. Polycarbonate). However, there are no current CRMs in the very malleable modulus regime (100 MPa down to sub kPa) needed for biological materials. Calibration of instruments for nanomechanical measurements requires calibrated force (spring) constant and tip shape and tip area function. ISO 11775 provides five methods to determine spring constant, but commonly used methods are not included and thus the standard needs a revision. One method is the Sader method based on resonant frequency and a look up value from data-sets for low force nanomechanical measurements. This is currently a qualitative technique and requires quantification via traceable low force methods in order to approach (5-10) % from the current uncertainty of at least 20 %+. The measurement uncertainty of AFM indentation depends to a large extent on the uncertainty of the tip area function of the AFM probe in use. Currently, manufacturers and end

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users do not have easy access to state of the art calibrated instruments. Following on this, standardised terminology in scanning probe microscopy (SPM) is also required so all users have a common basis. The ISO terminology in SPM ISO 18115-2 was first published in 2001 and although it has been republished several times since, it has not undergone significant revision. Over the last two decades, terminology has changed, and new modes developed. Hence a full revision of ISO 18115-2 is required. Current data analysis packages including models, algorithms and software for data extracted from AFM elastic properties, such as the Niget toolbox are for idealised samples and have limited applicability to biological materials. For the standardization of methods, a interlaboratory comparisons is required to take place (e. g. pre-normative VAMAS TWA2 - surface chemical analysis).

Once AFM nanomechanical measurements are validated, the data, methods, guidelines and recommendations, should be provided to ISO/TC 201 (Surface chemical analysis) for the standardisation of SPM determination of cantilever normal spring constants and the terms used in SPM. This will help on the revision of standards, such as ISO 11775 (Quantitative calibration of AFM force calibration), ISO 18115-2 (SPM terminology) and ISO 21222 (AFM visco-plastic measurement of biological materials). The results will also be presented to ISO/TC 164/SC 3 “Hardness testing” helping on the 019 ISO TC 164 mandate on “Traceability and Uncertainty improvement Instrumented Indentation Test (IIT)” [1]; and to CEN/TC 352 (“Nanotechnologies”) supporting the 003 CEN TC 352 mandate on “Traceability Improvement for Instrumented Indentation Testing” [2].

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 metrology research necessary to support standardisation in the nanomechanical measurements of soft materials including biomaterial and biological materials beyond the elastic region using atomic force microscopy.

The specific objectives are

1. To develop and select macroscale soft candidate reference materials (sCRMs) with elastic properties such as elastic modulus similar to those of biological materials including human organs ranging from 100 MPa down to 1 kPa. These sCRMs will feature high three-dimensional homogeneity with low aging effect, suitable for calibration and test of commercial and research-oriented nanoindentation instruments and bio-atomic force microscopy (bio-AFM).
2. To develop the necessary metrological work to update two ISO/TC 201 standards (ISO 11775 and ISO 18115-2): i) to develop a quantitative version of the commonly used “Sader method” to calibrate atomic force microscopy (AFM) cantilevers via traceable calibration of cantilever types using traceable low force methods; ii) to validate this quantitative method through an international comparison; and iii) to develop new and revised terms and definitions for scanning probe microscopes (SPM).
3. To develop a protocol suitable for future VAMAS international interlaboratory comparison with a generalised method for the traceable characterisation of the tip area function of sharp, flat punch and spherical AFM tips using the sCRM of objective 1. The focus will be for deep indentation depths >> 200 nm (nanoindentation) commonly used in biological measurements.
4. To develop a good practice guide and recommendations for the standardisation of data interpretation models, algorithms and open-source software for the extraction of the mechanical properties of biological materials using AFM elastic properties, nanohardness and complex elastic modulus. To perform a multi-scale comparison measurement campaign to validate these models, the software and the uncertainty budgets by using i) the developed sCRM (objective 1) and ii) traceably calibrated nanoindentation instruments, bio-AFM and microelectromechanical system based (MEMS)-SPM from objective 3.
5. To contribute to a revision of standards, ISO 11775, ISO 18115-2 and ISO 21222, by providing the data, methods, guidelines and recommendations, which are necessary for the standardisation of SPM determination of cantilever normal spring constants and the terms used in SPM, to ISO/TC 201. Outputs should be in a form that can be incorporated into the standards at the earliest opportunity and communicated through a variety of media to the standards community and to end users.

The proposed research shall be justified by clear reference to the measurement needs within strategic documents published by the relevant Regulatory body or Standards Developing Organisation or by a letter signed by the convenor of the respective TC/WG. EURAMET encourages proposals that include representatives from industry, regulators and standardisation bodies actively participating in the projects. The proposal must name a “Chief Stakeholder”, not a member of the consortium, but a representative of the user community that will benefit from the proposed work. The “Chief Stakeholder” should write a letter of support explaining how their organisation will make use of the outcomes from the research, be consulted regularly by the consortium during the project to ensure that the planned outcomes are still relevant, and be prepared to report to EURAMET on the benefits they have gained from the project.

Proposers should establish the current state of the art and explain how their proposed research goes beyond this. In particular, proposers should outline the achievements of the EMRP projects NEW05 MechProNo and IND05 MeProVisc, EMPIR project 19ENG05 NanoWires 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 1.0 M€ and has defined an upper limit of 1.3 M€ for this proposal.

EURAMET also expects the EU Contribution to the external funded beneficiaries to not exceed 30 % 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 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 biomaterial manufacturing, medicine and 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 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.