

Report from Workshop on Nanoindentation and Its Applications in Mechanotransduction and Characterization of Soft Materials

Workshop Summary

On April 1-2, 2015, at the Micro- and Nanotechnology Laboratory at the University of Illinois at Urbana-Champaign, the NSF nanoBio node hosted a workshop entitled “Workshop on Nanoindentation and Its Applications in Mechanotransduction and Characterization of Soft Materials.” Invited and contributed talks and workshop discussions were held with experts and students in the field of nanoindentation and AFM characterization of soft materials. The workshop focused primarily on nanoindentation, although mechanical characterization through force curves and other AFM-based techniques was a strong secondary focus. This Report summarizes the conclusions of the workshop discussions on the future of this field. The Report is divided into two parts: desired advances in the field of nanomechanical characterization of soft materials, and recommendations for development of resources which can advance the efficacy of the soft materials nanomechanical characterization community.

The Future of Nanomechanical Characterization of Soft Materials

Nanomechanical characterization of soft materials is a field full of promise, with plenty of horizons to be probed. The field faces significant challenges, as the techniques customarily used for nanomechanical characterization were tempered in the forum of hard, elastoplastic materials. This section can be considered as a wish list from the community for what improvements are most important for the field in the next few years.

1. Soft Material Standards

There are no universally-accepted soft material mechanical testing standards. Since nanoindentation uses indentations on standard samples to deduce the contact area between tip and sample—which is critical to obtaining accurate results—this is perhaps the most significant need in the soft material mechanical characterization field right now. For flat punch indenters, the contact area is constant with displacement (neglecting pile-up or sink-in, and presuming the indenter is deep enough in the material for tip—sample tilt to be neglected), but for other probe shapes, the contact area changes with indentation depth.

For engineering materials (metals, ceramics, etc.), calibration of the indenter tip on fused quartz is conventional and sufficient. Since the reduced modulus of fused quartz is 69.6 GPa and its hardness is 9.25 GPa and many of the materials of interest have reduced moduli and hardnesses in the kPa—MPa regime, indenter tips will penetrate much deeper into the soft samples than they do into the quartz calibration sample. This means that variations from the ideal indenter shape are not calibrated away for the entire penetration depth of the indenter tip. A proper calibration sample should have properties similar to those of the material to be tested. Measuring such soft or compliant calibration samples, however, shares a great deal of the same challenges as measuring the experimental samples themselves.

Some polymers have been proposed as calibration samples. These samples tend to age, however. This means that their properties change with time, which is particularly troublesome for researchers new to the field or smaller groups which may not have the resources to obtain fresh material or to microtome the samples to refresh the surfaces.

Most proposed soft material standards need to be freshly prepared by the researcher to avoid this aging problem. This relies on an assumed consistency of preparation, which is perhaps not the safest assumption, particularly when done by researchers with little experience in preparing such samples.

2. Low Loads, Large Displacements

Most conventional nanoindenters are designed for few-micron displacements. Microindenters can go to larger displacements but generally lack the ability to apply low loads. Soft material measurements require high sensitivity to low loads, and they generally require large displacements to obtain a reliably measurable load. Additionally, rough materials (rough surfaces or materials with a roughness due to porosity) require deeper indents in order to obtain a consistent contact area with depth, and viscoelastic materials may creep a great deal over the time of the indent. Both of these factors lead to a need for large displacements.

Most researchers in the field use instruments which are designed for the μN — μm load—displacement regime. The desired approximate range is closer to 10s of nN — mm .

3. Non-ambient Temperature Testing

Many interesting changes in mechanical behavior are temperature-dependent; for example, changes in mechanical behavior of polymers below and above the glass transition temperature or in composite or biomaterials at low temperature as their included water begins to freeze. In addition, it would be desirable to do time-temperature superposition experiments with dynamic nanoindentation.

Unfortunately, thermal drift in most commercial nanoindentation systems is so dominant that data taken at temperatures significantly different from room temperature is unreliable. Creating hardware which can maintain the tip and sample both at a uniform temperature—so that thermal expansion or contraction of the tip and sample does not occur appreciably as a function of contact area between the tip and the sample during the indent—would greatly relieve this problem.

4. Surface Detection and Sample Preparation

Sample surface characterization is key to getting reliable results and straightforward experiments. By their nature, some samples are difficult to form into something approximating the idealized perfectly flat surface assumed by some analysis models. Models exist which account for adhesion, such as the DMT model, but unknown surface roughness is difficult to account for, which can be a problem for porous samples.

Samples which are hydrated in their natural state generally change their mechanical properties upon drying, and sometimes this change in properties is not recovered upon rehydration of the sample. Samples such as this should therefore be kept hydrated. Operating in fluid requires further consideration in many cases, due to hydrodynamic drag on the tip during the AFM force curve or the indentation, as well as changes during the indent in forces on tips which are not fully submerged.

Surface detection is a particular problem for samples which must be kept hydrated but which dissolve slightly into the water at the surface, such as many hydrogels. In such a case, the surface is not at a distinct location, as it would be for a submerged material such as bone, but instead forms a gradient of material between the sample bulk and the fluid. This makes it difficult to determine the location at which the tip should be considered to contact the surface. While using flat punch tips can avoid the severe ramifications to the results of using tips whose contact area with the sample varies with penetration depth into the sample—which is difficult to define for samples with no clear surface and will give incorrect results for experiments done with the tip unknowingly already buried in the sample—there also should be a zero-force point in the data in order to discern which properties of the load—displacement curve are due to the sample and which are due merely to the fluid. When faced with ambiguous surface locations, which can be exacerbated in samples which also display a great deal of creep, the indenter can falsely engage above the surface and extend to its full displacement range without probing the properties of the true sample.

5. Combined Instrumentation

Nanomechanical characterization by itself is a powerful technique for understanding materials. This can be enhanced by including simultaneous or near-simultaneous observation of other properties or behaviors of the samples. Historically, optical microscopy, profilometry, or AFM have been used to observe pile-up, sink-in, or fractures in hard materials after indentation. With soft materials, in which the indenter may not leave a significant residual impression, the complementary observational techniques may differ. Some soft materials are too adhesive for AFM imaging, shear too readily for stylus profilometry, and are optically transparent. Furthermore, many soft—especially biological—materials are locally inhomogeneous. It is important for accurate characterization of such materials to identify where the indentations were made and whether the indentations included more than one distinct region. Complementary observational techniques should ideally be capable of imaging the indentation sites directly, either simultaneously with the indent or after the indent with a calibrated offset between indent and image, as residual impressions from indentations in soft materials are often difficult to find after removing the sample from the instrument.

6. Emerging Materials of Interest

Soft composite materials represent a field of study where nanoindentation is poised to be a particularly well-suited characterization technique, since local differences in mechanical properties can be probed using the high lateral resolution of this technique. However, in addition to the measurement challenges for soft material nanoindentation discussed above, materials with local mechanical inhomogeneity may require further consideration, particularly if indents are made at borders of regions with differing mechanical properties. For example, pile-up or sink-in behavior affecting the contact area between tip and sample may be different for the harder and softer areas of the sample in contact with the tip. Depending on the differences between mechanical properties of local areas within the composite, AFM-based experiments may require extra care in cantilever selection to ensure that the cantilever selected is not too stiff to measure the compliant areas of the sample or too compliant to measure the stiffer areas of the sample. Sample surfaces may also be more difficult to prepare.

Biomaterials are of immense interest in science today. Understanding the roles of forces and mechanical properties in determining the behavior of biological systems is becoming increasingly important in biophysical studies, particularly as they relate to disease on the cell or tissue level.

Development of a robust, simple set of mechanical characterization assays would aid in standardizing such measurements so results can be more readily compared across the vast range of biological samples under study. Furthermore, given the significant alterations in mechanical properties of fixed versus unfixed samples, the role of fixation should be addressed head-on when developing these protocols.

Soft Material Nanomechanical Characterization Shared Efforts

Researchers new to the field of nanomechanical characterization of soft materials are entering the field and making contributions in increasing numbers. Their backgrounds are mainly in other characterization techniques of soft materials or in nanomechanics of non-soft materials. For these researchers, deep delving into the involved details of the field may not be desirable, as nanomechanical characterization may be only a small part of their material characterization program. They often do not have extensive connections within the soft material nanomechanics community. Many of these researchers find themselves either spending a considerable amount of time rediscovering techniques and caveats the community already knows, or performing naïve experiments and analyses based on incorrect assumptions. It is a role of the community to welcome these new researchers into the soft material nanomechanical characterization field by providing them with tools and guidance to save them time and confusion. This section explicates ways in which the community can improve the efficiency of non-expert research groups in this field.

1. Analysis Routines

Most popularly-used nanoindentation data acquisition software provides basic Oliver—Pharr analysis. This is appropriate for elastoplastic materials, but it often does not describe soft materials, which tend to be highly viscoelastic, poroelastic, or poroviscoelastic. For this type of analysis, researchers must acquire appropriate analysis routines, whether by writing their own analysis code or by purchasing commercial analysis software.

Research groups which routinely work in the field of nanomechanical characterization of soft materials tend to develop internally-written analysis routines, which are shared with collaborators. As the field of nanomechanical characterization of soft materials expands, however, more research groups begin to work in this area. These new groups may not have collaborations with established groups, which limits their access to such advanced, more sample-appropriate analysis tools. Furthermore, researchers new to the field may assume that elastoplastic analysis is sufficient.

To this end, it is suggested that experienced research groups in the field of soft material nanomechanics make publically available their analysis code so other groups can avoid duplication of effort. In order to prevent this from becoming a push-button black box, it is also suggested that the code be accompanied by tutorials instructing new researchers on how to choose the correct model or models, as well as letting them know what to expect from the analysis and where the models become less reliable.

2. Instructional Workshops

As many new research groups move into the field of soft materials nanomechanical characterization, both from the bio- or soft materials side and from the traditional engineering materials side, some forum should be provided in which to share best practices. Many researchers new to the field find

themselves isolated, as nanomechanical characterization of soft materials is still a comparatively small community, and they must improvise solutions to thorny measurement and analysis problems. Since there is a comparatively small number of experienced researchers to share their expertise compared to the number of researchers entering the field, it would be useful to disseminate the hard-won experience in the field to new researchers by means of best practices workshops.

These tutorial and best practices workshops would combine forthright discussion of limitations of existing techniques with expert advice on how to test difficult samples, ideally with instruments present in order to run samples as demonstrations. The aim would be to provide new entrants to the field with a starting point and realistic cautions, plus familiarizing them with the resources and researchers available to help them in their research.

It is key to provide detailed guidance to new or isolated researchers on how to test soft material samples well, as most of the extant guidance in the literature is designed for hard materials, which have different challenges. The best-implemented analysis routines will not rescue a poorly-executed experiment. Significant improvements to the efficiency of research and to the production of reliable-quality results can be made by disseminating thorough guides to the pitfalls and practices of soft material nanomechanical testing.

3. Interdisciplinary Meetings

While this workshop focused on nanoindentation and AFM-based nanomechanical characterization, soft materials are often characterized with other techniques which measure similar quantities but with substantially different instrumentation—for example, optical tweezers are often used for force measurements in the bioscience community. Probe-based mechanical testing has different practical considerations—for example, contact area between probe and sample is a significant consideration—but more thorough comparison of techniques and mutual education between the fields can be of use in finding alternate perspectives to solve problems.

The ability to test samples with a range of different nanomechanical or micromechanical characterization techniques can build confidence in the reliability of results. More importantly, multi-technique testing programs can point out discrepancies between results from different techniques, which can lead to more acute analysis of the differences between and uncertainties inherent in each of those techniques.

4. Materials Databases

A repository of example data could assist in identifying optimal testing and analysis procedures for unknown materials, perhaps also leading towards development of soft material standards by including tests on the same type of material from different research groups. Such a database would include examples of experimental characterization for a range of materials, including the raw data, methods used—such as load functions and tip geometries—and subsequent analysis. Other researchers with materials similar to those in the database could then be inspired by the protocols used to test the database materials and more easily develop or adapt strategies for testing their materials. Such databases would also enable theorists to have access to experimental data for developing or testing new models.

Report Summary

While some longed-for developments in the field of soft material nanomechanical characterization await further advances in instrumentation—such as low load, large displacement testing and reliable temperature-dependent testing—improvements can be made to practices in the field. Communication among experts and newcomers to the field is crucial in enabling incoming researchers to make valuable contributions efficiently and to correctly characterize their materials according to current best practices in testing and analysis. This communication should take place both by increasing availability of online resources such as tutorials and fitting or modeling tools and by holding interdisciplinary workshops to evaluate needs and share expertise among practitioners in the field of soft material nanomechanical characterization.