Perspectives in Scanning Probe Microscopy from the 2021 Joint International Scanning Probe Microscopy and Scanning Probe Microscopy on Soft and Polymeric Materials Conference

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In March of 2020 we were well on our way to organizing and hosting one of the premier scanning probe microscopy (SPM) conferences in Breckenridge, Colorado, USA. For the first time, the meeting would synergistically combine International Scanning Probe Microscopy (ISPM) and Scanning Probe Microscopy on Soft and Polymeric Materials (SPMonSPM), to increase the breadth of audience expertise and experience. We coined the joint conference iSPM^3 – a moniker we hope will persist in future events. The plan for 2020 iSPM^3 screeched to a halt in mid-March, amidst the COVID-19 pandemic. Luckily, we were able to regroup and reimagine the conference for 2021 as a virtual experience. The conference took place June 28th to July 2nd with a mix of live and pre-recorded content. Spread throughout the week were 4 live panel discussions, 3 plenary talks, 23 invited talks, 75 contributed talks, 15 posters and 3 social events. The technical program represented much of the latest and most impactful research in the field. The panel discussions sought to step back from specific research findings and assess the state of SPM as it pertains to some of the most pressing opportunities and influential successes. The lively discussions painted a vibrant future for SPM in both breadth and detail, and we hope that similar discussion can be a mainstay of future SPM conferences. Here, we attempt to distill some of that discussion to motivate researchers across the field of SPM in the coming years.

Background on the conferences

ISPM

The International Scanning Probe Microscopy (ISPM) Conference began in Seattle in 1999 being championed by leaders in the SPM community. This began over 2 decades of annual conferences held in a rotating fashion acrossEurope, Asia, North America and South America, bringing together young aspiring researchers with established leaders in the field, including some of the inventors of the AFM themselves. While traditionally a strong focus of ISPM was applications of SPM for life sciences, the conference has consistently sought to include researchers working on the development of all SPM techniques, as well as users of SPMs from all scientific and industrial disciplines such as materials science, basic physics, semiconductor industry, and energy industry. ISPM^3 2021 was the 22nd iteration of ISPM.

SPMonSPM

The Scanning Probe Microscopy on Soft and Polymeric Materials (SPMonSPM) conference began as the SPM on Polymers conference in Santa Barbara, CA, USA in 1999. It continued for 2 more iterations in Europe on a biennial basis, with an interruption from 2003 to 2012. The major topics considered during these conferences were mainly focused on polymeric based material morphology and nanomechanics, organic electronics (solar cells, field effect transistors, batteries, …), and 2D molecular self assembly to name a few. The conference was reimagined to be more inclusive of (biological) soft matter and adopted its current name in Kerkrade, NL in 2012. It has now been hosted on 3 continents, and it is a premier venue for reporting state of the art advances in soft matter SPM. ISPM^3 2021 was the 5th iteration of SPMonSPM.

Perspectives from the panels

The panel discussions covered 4 topics at the forefront of SPM advancement: (1) AFM for Soft and Bio: Latest advances and future prospects, (2) Quantifying Functional Properties: Can you really measure *X*?, (3) Frontiers of Artificial Intelligence and Machine Learning in SPM, and (4) Looking forward: Future opportunities and needs for SPM instrumentation, software, training and community. The panelists represented a diverse representation of the field, at numerous stages throughout their careers from students to SPM pioneers. They included academics, industry representatives and national laboratory scientists. The panelists and affiliations are listed in table 1. The panels alternated between prepared questions, audience questions, audience polls and open discussion. Overall, the discussions were an opportunity to celebrate the many successes of SPM, while painting a rich path for future development. Full recordings of the panels are available at: https://www.nist.gov/news-events/events/2021/06/2021-international-scanning-probe-microscopy-ispm-scanning-probe

Table 1: ISPM^3 panelists and affiliations

AFM for Soft and Bio: Latest advances and future prospects	**Quantifying Functional Properties: Can you really measure *X*?**	**Frontiers of Artificial Intelligence and Machine Learning in SPM**	**Looking forward: Future opportunities and needs for SPM instrumentation, software, training and community**
Johanna Blass *(Leibniz Institute for New Materials, Germany)*	Samuel Berweger (*National Institute for Standards and Technology, USA*)	Gabriel Gomila (*University of Barcelona, Spain)*	Rachael Cohn (*Cornell University, USA)*
Ricardo Garcia (*Consejo Superior de Investigaciones Cientificas, Spain)*	Charles Clifford (*National Physical Laboratory, UK*)	Sergei Kalinin (*Oak Ridge National Lab, USA*)	Georg Fantner (*École polytechnique fédérale de Lausanne, Switzerland*)
Alice Pyne (*University of Sheffield, UK*)	Neus Domingo (*Institut Catala de Nanociencia i Nanotecnologia, Spain*)	Igor Sokolov (*Tufts University, USA*)	Takeshi Fukuma (*Kanazawa University, Japan*)

Simon Scheuring (*Weill Cornell Medicine, USA*)	David Haviland (*KTH Royal Institute of Technology, Sweden*)	Rama Vasudevan (*Oak Ridge National Lab, USA*)	Rajiv Giridharagopal (*University of Washington, USA*)
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AFM for Soft and Bio: Latest advances and future prospects

Biology has been a major application area of SPM since the early days of the field. Biological systems present unique challenges and opportunities for the microscopist as the samples tend to be fragile and dynamic, spanning length scales from single proteins to multi-cell assemblies, and often require excellent force precision to avoid sample-damage and sense desired phenomena. These demands have led to countless engineering innovations and related scientific discoveries.

Our panelists in this topic had expertise in single molecule force spectroscopy, high-speed AFM, cellular nanomechanics and high-resolution molecular imaging. The panel began with a discussion of the major microscopy breakthroughs in biological SPM and the new science they enabled. The panelists covered 4 major breakthroughs: phase imaging and its ability to enhance compositional contrast, single molecule force spectroscopy and the insights it provides into protein folding and binding, force-volume mapping for its probing of mechanical and chemical interactions across cells, and high-speed (HS) -AFM for its unique simultaneous measurement of biomolecule structure and dynamics. HS-AFM was expanded upon to mention its ability to provide molecular insight without the need for averaging. The panelists applauded the work of Toshio Ando to reveal the walking mechanism of Myosin V, which was unproven prior to the advent of HS-AFM. HS-AFM was touched upon repeatedly throughout the discussion and brought numerous questions from the audience. Despite polling showing that only a small fraction of audience respondents were currently using AFM or HS-AFM to study dynamic processes, innovation in HS-AFM still dominated the Q+A and points to the continued growth in the sub-field. The future of HS-AFM instrumentation drew mixed opinions between a need for highly-specialized instruments that would examine ~100 nm regions quickly compared to larger area scanners that could cover the gamut from proteins up to cells. Hybrid approaches were also discussed wherein slow, large scanners are combined with fast, small scanners. Another opportunity area is integration of functional property mapping such as nanomechanics with HS-AFM. Such an approach demands a rigorous understanding of the cantilever dynamics to interpret e.g.

nanomechanical signals, and is an active area of research. HS-AFM also led to a compelling discussion of time-scale correlations between computer simulations (e.g. molecular dynamics, density functional theory) and AFM imaging or force sensing. On the one hand, it was noted that these correlative studies are happening successfully right now, but on the other hand it was raised that we don't necessarily need to match every aspect of the experiment and simulation. Questions arose, such as how close in time scale the approaches are, and how much of the experimental setup should be simulated (e.g. tip apex vs. entire cantilever). There were no definitive answers on these questions, but it was noted that even when time scales mismatch and the experimental setup is overly simplified in the model, it is still possible to gain considerable insight from the complementary nature of simulations and AFM measurements, even beyond HS-AFM.

A second recurring topic of the biological SPM panel was the need for a unified community in biological SPM. This discussion originated early in the panel when considering the role that SPM played in understanding the COVID19 pandemic. The panelists noted some key works such as David Alsteen’s group’s study of SARS-CoV-2 protein-to-receptor binding. However, the comparatively small number of impactful works versus other methods suggested that the SPM community needs more organization. For non-specialists, SPM is not at the forefront of methods for biologists, thus they gravitate to more familiar methods. SPM still has to significantly mature to reach the level of adoption of optical microscopy, electron microscopy, or crystallography. A more unified community would develop standard workflows that could be applied across numerous institutions compared to the current approach where each research group maintains their own work flow for sample prep, instrument operation and data analysis. Analysis of biological SPM data sets in particular was called out by panelists and audience poll-respondents as an area that needs considerable development and dissemination. Currently, biological SPM data analysis is hampered by the numerous proprietary file-formats existing for data produced by different commercial and home-built machines. These proprietary formats limit the direct sharing of analysis codes. Likewise, widely adopted processing and visualization tools are still lacking, although tools such as Gwyddion, which afford user add-on toolboxes are making headway. Machine learning and AI is one possible path forward for handling the large and complex data-sets produced in emerging biological SPM experiments, but we still need methods of establishing ground-truth for teaching those methods. If SPM is to have broad impact in clinical medical use, these standardized workflows will be even more essential.

Quantifying Functional Properties: Can you really measure *X*

While standardized methods and analysis were a key discussion point in the biology panel, the concept was expanded considerably in the “Quantifying Functional Properties” panel. Experts sought to answer the question of how well we can really measure a given property, be it topographic, mechanical, electrical, etc. A question that arose in the panel was whether quantification meant just attaching a number to something, or whether it was implied that quantitative property measurements met some threshold level of accuracy and precision. Although it may be a question of semantics, achieving consensus on what it means to quantify is foundational to future high-veracity SPM measurements.

The panel started out discussing arguably the most basic properties addressable with SPM: force and topography. Even there, caveats still exist. For force, the panel felt that established methods were suitable for describing tip sample force during lower-frequency operation based on progress in spring constant calibration (which is currently bolstered by activities such as the Global Cantilever Initiative) and optical lever sensitivity calibration. Nonetheless, extension from the quasistatic, low frequency regime towards more complex dynamic operation with higher modes and multifrequency operation is still an active research area. Likewise, for topographic mapping, the established methods are excellent at quantifying the height of stiff materials, but the community rarely applies methods to accurately account for the shape of this tip and its influence on lateral structures. Application to soft materials is much more difficult, and clearly demands that assumption be made regarding the motion and deformation of the sample itself.

Moving on to nanomechanical measurements, the field is still quite open and exciting. Audience polling showed that nanomechanical properties are the most sought quantitative property measurements in SPM. At their core, nanomechanical measurements demand precise knowledge of the probe geometry and composition, not just as fabricated, but throughout the experiments. Fouling and tip breakage undermine even the most careful measurements. The panel had differing views on whether the hitherto approaches for nanomechanical characterization were heading in the right direction. Considerable progress in matching nanoscale and bulk properties has been made with techniques such as nano-DMA when combined with clean, well-defined-shape tips of sufficient radius to not induce plastic deformation. However, panelists still questioned whether the need to match AFM properties of modulus to their bulk analogues, or if the focus should be on catching some of the intrinsic nanoscale phenomena that aren't captured in a single bulk quantity. Clearly, the ability to compare properties between different probes and other complementary instruments at different length scales adds value to characterization, but there may also be value in adapting to interpret more mechanical descriptors of the tip sample interaction.

Shifting from mechanical interactions to electrical interactions leads to even more need for characterizing and modeling the tip sample interaction. Whereas the mechanical interactions are highly localized at the tip apex, the electrical interactions influence the entire tip and often the cantilever as well. Quantification of intrinsic electrical properties requires accurate modeling of these interactions. Furthermore, whereas mechanical characterizations can often leverage reference samples to improve accuracy of measurements, it is much more difficult to wire and install an electrical reference sample and then replace it with an unknown sample assuming the same circuit properties exist. Electromechanical properties such as piezoelectric coupling coefficients, present the additional challenges of combining the intricacies of electrical and mechanical measurements. Considerable progress has been made in quantifying electromechanical strains e.g. by replacing the optical beam deflection with interferometric detection; however, the electric fields within the sample are still largely immeasurable and require assumptions and modeling.

Broadly, the audience and panel felt that standardized methods and workflows were an essential step forward for quantitative SPM. This must be coupled with quantified accuracies and uncertainties that attempt to propagate both experimental and epistemic errors. There is strong demand for reference samples that reflect the full range of properties SPM seeks to quantify. Such samples can be used to reduce uncertainties and refine new methods, as long as challenges in implementing such samples are addressed. Overall, the progress made in SPM away from simple contrast and towards intrinsic property measurement is commendable, but there remains considerable work ahead to make these measurements more robust, eliminating intrinsic measurement artifacts, and improving user experience and accessibility to a broader cross-section of SPM users. The enterprise towards quantitative AFM requires a joint effort from all the vertex of the community, i.e., users, scientists and manufacturing companies, to think out of the box and revisit technical designs of both, AFM hardware and AFM tips to advance towards optimized tools with broader applicability and higher market potential to improve its penetration into the scientific and technical sectors, afterall enhancing the critical mass of end users.

Frontiers of Artificial Intelligence and Machine Learning in SPM

The veracity of the data captured by SPM also played a key role in the next panel covering the rise of applications of artificial intelligence (AI) and machine learning (ML) in SPM. Throughout the week, it became abundantly clear through the many talks and posters focusing on the topic, that the community is eager to capitalize on the recent advances from the fields of machine learning and AI. Indeed, 50 % of poll respondents indicated that they already used ML in their research, while 90 % of poll respondents indicated they would like to in the future. Following efforts in the electron microscopy community, many groups within the SPM community are now actively exploring possibilities to enhance AFM capabilities, and ultimately our understanding of material properties, through adoption of such methods. Our panelists included a well-versed group of SPM experts who were chosen as they have been actively applying, and in some cases developing, AI and ML methods in their SPM research.

During the discussion, the panelists identified several areas where SPM could benefit from ML/AI methods. These range from ways to improve the microscope operation and user experience, to autonomous experimentation, or as an aid for interpretation and analysis of complex datasets. Particularly promising is the utilization of ML/AI for lowering the operation difficulty of an AFM instrument. Indeed, ML is particularly well suited to help overcome many routine experimental processes currently necessary for AFM operation (e.g. cantilever tuning, feedback gains, laser alignment). As an extension of this, we are likely to see a rise in applications of ML/AI for automated experiments, although it is unlikely that the AFM operator will be left out of the loop anytime soon.

Next is the possibility of utilizing ML/AI for scientific discovery which is of particular interest to the community, but comes with extensive challenges. As is the nature of the AFM technique, and in line with the previous panel, the panelists agreed the goal of any analysis method should be the extraction of a quantitatively reliable map of a structural or functional property from the measured observable. Unfortunately, AFM often represents an indirect measurement of a material property, and often requires a theory to bridge the gap between the measurement observable to a functional material property of interest. This, along with the fact that we often lack a good understanding of all underlying experimental factors (e.g. tip condition, ambient water layers, adsorbates, measurement artifacts, instrument noise) can complicate immediate interpretation of the results coming from the microscope. In cases where we have a solid theory, and a good grasp or control of underlying factors, ML can be extraordinarily useful in accelerating and enhancing our understanding. In particular, in instances where numerous possible models or theories exist (e.g. mechanical force curves), we can and should leverage recent advances in Bayesian statistics to enable probabilistically determined model selection (i.e. Bayesian model selection). On the other hand, for measurements which lack a fundamental physics based model, or involve unknown underlying experimental factors, we need to be very cautious in our adoption of these approaches.

One of the major problems of analyzing AFM images with the ML methods is the repeatability of the AFM images. In part, this lack of veracity is caused by poorly defined physical mechanisms responsible for the creation of the AFM contrast. Furthermore, the contrast might be altered by the factors that are hard or impossible to control, such as the precise radius of the AFM probe, small variations in the load force due to imperfect feedback, temperature fluctuations, etc.

It is worth noting that applications of machine learning to AFM are early in the developmental stage. In addition, the ML area by itself is still far from achieving the mathematical rigor of classical statistics. As a result, conclusions obtained with the help of machine learning are not always statistically significant. It is expected that ML methods will be further developed and adopted for specifics of AFM.

Looking forward: Future opportunities and needs for SPM instrumentation, software, training and community

Our last panel discussion was a broad forward looking panel on the opportunities and needs of the SPM community. The panel included a diverse range of researchers at various levels of their scientific careers. Overall, there was broad enthusiasm for future advances in AFM. As has been previously mentioned, the commercialization of high speed AFMs has been a paradigm shift for video rate imaging of self assembly dynamics and biomolecular processes. High speed AFM also brings opportunities for extending commercial AFMs to the 3rd dimension, by either mapping solid liquid interfaces or buried structures by means of tomographic imaging. Presumably, video rate functional mapping when available will extend high speed AFM to other areas of material and energy sciences. The panel were particularly optimistic about continued progress in the area of correlative microscopy, such as combining AFM with optical probes(e.g. infrared, Raman) enabling chemical information to be inferred via AFM force detection. Equally, it was stressed that combining microscopy methods, in which AFM plays a supporting role to existing methods (e.g. analytical scanning electron microscopy + AFM), can have an equally important role to play in correlating chemical and physical material properties. Indeed, the ability to correlate chemical information with the plethora of already available functional (e.g. nanomechanical, electrical) channels will further strengthen AFM. Generally speaking, such correlative AFM can still be considered in its infancy, and further commercialization will likely continue to reduce the barrier to entry and make these methods more widely adopted.

Regarding commercial AFMs, there was plenty of discussion of what are the needs of the AFM community and if they are being met by the vendors. While the quality and sensitivity of the commercial AFMs have improved remarkably, this has come with a significant price increase. The increased complexity of today's AFMs can have the simultaneous effect of stifling innovation, as it becomes more and more difficult to modify these newer microscopes. This is in stark contrast to the early 90’s, widely seen as the most innovative era of AFM development, in which most of the innovation took place on home-built systems which were readily adaptable. In this regard, base model AFM instruments which are open and programmable are very attractive for SPM technique developers. The panel also debated whether it was best in some situations to have a cheaper base model AFM that does one thing really well, as opposed to an expensive instrument that can do everything well. The idea of modular AFMs which could have their capabilities expanded overtime also gained widespread panel support.

Regarding instrumentation, it's remarkable that the base AFM technology has not changed much since the early days since its invention. Many of the main components of early AFM instruments (e.g. optical beam detection, PID feedback, piezo actuators) are still standard in most commercial AFMs. That said, looking forward, with the progress made in programming tools which make it easier and more straightforward to control hardware at a lower level (e.g. field programmable gate arrays), this brings forward an opportunity to improve on existing analogue controls, enabling more advanced and possible automated real time optimization of imaging parameters. This would likely be accompanied by an easier operator experience, which is a current limitation for more advanced AFM imaging modes. For the SPM developers, the ability to have this level of control via integrated software development kits would likely spur innovation in the field further.

Another area of deep discussion was the topic of open source science, including data, software and hardware. Our audience poll indicated that the community is very optimistic (72 %) of open source efforts. One challenge here is the various proprietary hardware, software and data formats used by the AFM vendors. It was noted that community-led open source efforts on the analysis sides are being developed. As pointed out by Takeshi Fukuma, progress will likely require a consortium of researchers from across the AFM community (including academics, industry, students etc.) to come together for the standardisation of collection and analysis methods, data structures etc.

On the topic of ease of use, there was some debate about how user-friendly modern AFM instruments are for the broad research community. Certainly, with the improvement in measurement technology and automation, specific tasks are becoming easier (e.g. aligning laser, probe calibration). However, in the realm of microscopy methods, compared to optical or electron microscopy methods, AFM is still seen as being more challenging to obtain research-grade data from. Even the analysis of AFM data can be considerably more complex, and often impossible to do so meaningfully, if the tip-sample interactions and measurement conditions are ill defined. The panel largely agreed that AFM operation still requires a base-line level of expertise, and operation involves tips and tricks, and even some level of operator intuition. Several options for improving the ease of use were discussed, ranging from organizing more training workshops for students, increasing the number of talented and trained AFM experts and more knowledge transfer. Drawing from the AI/ML panel there is also hope that their implementation by the AFM vendors may help remove some of the more monotonous, but equally important aspects of AFM operation.

Last but certainly not least, there was broad support within the panel and audience that as a community we should have some sort of AFM-based scientific society. As such, we believe the continued amalgamation of iSPM[3] may help to fill the role, by regularly bringing SPM leaders, developers, practitioners and students together under one common roof. At the same time, it is equally important that we continue to represent SPM at application-specific conferences, in order to disseminate the many great endeavors and novel nanoscale information accessible by modern SPM technologies and methods.

Conclusions

Overall, the week's discussions show a rich future for SPM with ample opportunities for growth and impact. We continue to see major advances in the field of SPM, particularly related to high resolution and high-speed AFM, dynamic AFM for functional property mapping, 3D AFM, correlative AFM, and AFM for chemical imaging. Future adoption and application of ML and AI will allow for improved ease and accelerated discovery concerning both data analysis and measurement automation. We still have many challenges facing us concerning reliability and quantitativeness of methods, particularly when we consider the non-expert SPM user. At the same time, this challenge will likely spur the next round of improvement of functional mapping methods and hopefully remove underlying measurement artifacts. As a community, we should continue to engage closely amongst ourselves, with the AFM vendors, and the application specialists. Importantly, we must continue to support venues such as these panel discussions to encourage the open sharing of new SPM ideas and continually update goals and vision for the community.

Further reading

Biological SPM

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