Exploring the nano-world of plant cells with hybrid photonic-mechanical forces

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The ongoing effort to achieve high-resolution nondestructive nanometrology for material characterization has recently generated many exciting venues in the domains of multi-frequency and multi-excitation exploration. An example is the newly developed hybrid photonic-nanomechanical force microscopy (HPFM), which through its chemical, mechanical, and subsurface measurement capabilities furnishes a window into the nanoscale properties of plant cells. While the approach has been demonstrated successfully to obtain simultaneous chemical and morphological information from cross sections of Populus deltoids, the path to a complete characterization is not yet one without difficulties mainly due to inadequate specific instrument development. Nevertheless, in light of new observations and discoveries in the probe-sample system dynamics, it is possible to lift this inadequacy by invoking specialized peripheral instrumentation. Here we address how we may experimentally characterize plant cells by capitalizing on multiple mechanical and optical sample excitations in a hybrid fashion.

Introduction

The desire to observe matter at ever-smaller details and scales possible so as to resolve its deepest fabric has always been a driving force in the exploration of the physical world. From the early visual inspection to the modern sophisticated detectors, material characterization has invoked a variety of tools operating at different length scales. Photons and electrons, through reflection, refraction, transition and tunneling can probe the physical and chemical properties of many materials and have provided the basis for some of the most powerful investigative and exploratory microscopy techniques. In addition, more recently, scanned probes have opened up new possibilities for obtaining physical and chemical properties of materials, some of which not amenable to direct studies by photons and electrons, thus broadening the class of materials that can be examined. However, two major challenges continue to prevail, albeit progress continues to be made in overcoming them: non-destructive high-resolution subsurface probing capability and high spatial and spectral resolution chemical mapping. In a recent study supported by the BioEnergy Science Center (BESC) at the US Department of Energy's Oak Ridge National Laboratory, it was demonstrated that simultaneous mechanical and chemical mapping at high resolution is feasible using the hybrid photonic nano-mechanical force microscopy (HPFM). HPFM, introducing a new and general modality that contributes to overcoming the challenges associated with both spectroscopic and microscopic characterization of a broad class of materials, was demonstrated by studying the effect of a sequence of chemical processing of biomass. The processing is under study to facilitate a more effective release of sugars that can be converted to biofuel through delignification.

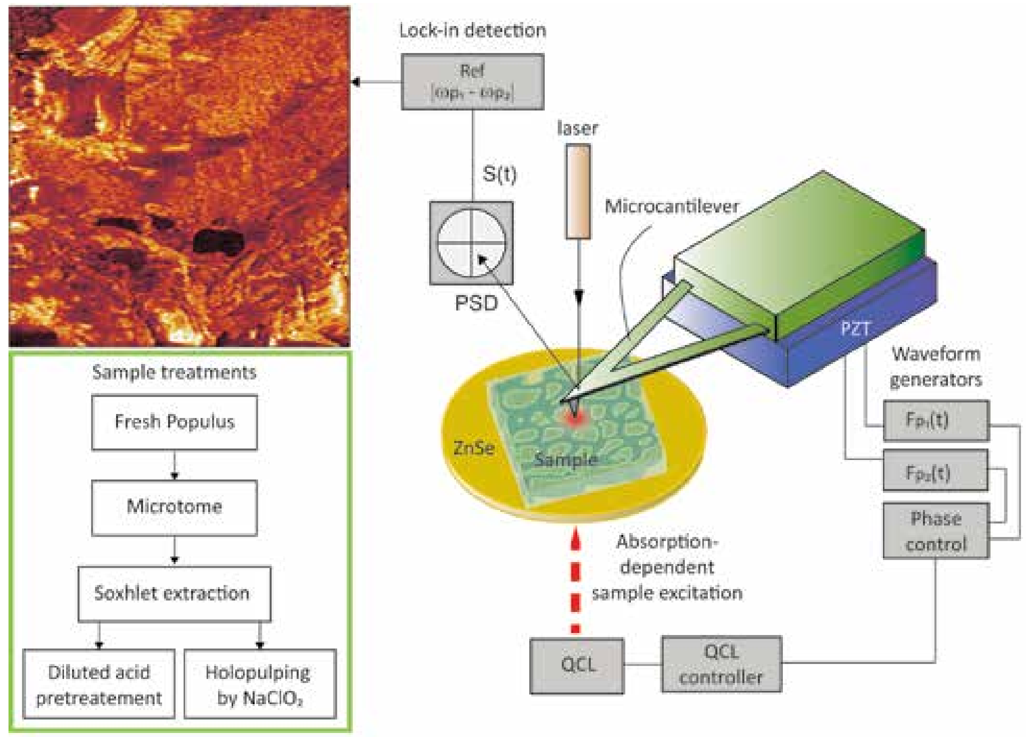

Fig 1: *Setup of hybrid photonic-nanomechanical force microscopy (HPFM). The non-linear force between the probe tip and the sample allows mixing of the forcings on the probe (F_{p1} and F_{p2}) and the forces induced by the modulated spectral absorption of the QCL beam. This highly sensitive actuation system is used for the investigation of Populus cross-sections at various chemical treatment stages designed for biomass ethanol production.*

Plants, in all its diversity, are ubiquitous in our planet with forests covering an estimated area of 32,688,000 km^2 [Hansen *et al.* (2010)]. Participating through photosynthesis, oxygen consumption, and carbon dioxide emission, the various plant populations not only control the climate and vital signs of life on earth but also provide feedstock for biofuels. Recent dual efforts to boost energy efficiency and renewable energy have rendered biomass feedstock as an important green source for large scale biofuel production. An important element in the multidisciplinary scientific research undertaken for an efficient conversion of non-food plants to biofuel is an understanding of the effect of the individual chemical processing steps that would break down the plant cell wall and release the sugars contained therein. Therefore, nano-scale information on both structure and organization of the various organic materials making up the cell walls is necessary. Measurement of the plant biological material properties at the nanoscale, that is, nanometrology of plant cells, is however no straightforward task. What is certain is that the question of exactly how one may go about carrying out meaningful nanoscale measurements, that is, obtaining a complete set of self-consistent quantitative results on the physical and chemical properties of given plant cell walls not only cannot be currently fully answered, but also cannot be generated from a single instrument. Quantitatively, however, employing an array of instruments, one could investigate various relevant cell properties such as morphology, subsurface features, lignin and cellulose content, and elasticity. Therefore, instruments that can perform mixed measurement modalities are highly sought after.

Hybrid photonic nano-mechanical force microscopy

The atomic force microscope (AFM) [Binnig *et al.* (1986), Herruzo *et al.* (2014)], in its traditional form, is a practical member of the larger class of scanning probe microscopes (SPM), offering a versatile platform for the study of plant cells at the nanoscale. Innovative measurement modalities of the AFM are however required to characterize plant biological materials beyond topographic mapping. In this article we discuss how simultaneous mechanical and optical excitations of samples of biomass can provide new information on the chemistry and morphology of plant cell walls.

In AFM, in addition to pure contact mechanical forces, several forces due to presence of electric charges, carrier generation and light scattering, capillary forces, and thermo-mechanical forces can be involved in the generation of a signal. Each of these forces can offer different or corroborative views of the sample. Under strict environmental control, a collection of the atoms making up the cantilever probe tip interact via van der Walls forces with a collection of sample atoms immediately underneath the apex of the tip. This force, being nonlinear in its spatial dependency, causes or "synthesizes" oscillation components at frequencies other than those explicitly involved from excitation frequencies. This is the hallmark of many nonlinear phenomena and can be observed in other branches such as nonlinear optics, spectroscopy and plasmonics and is the basis for the operation of mode synthesizing atomic force microscopy (MSAFM).

In hybrid photonic-nanomechanical force microscopy (HPFM) [Tetard *et al.* (2015)], the sample is exposed to modulated infrared light, whereupon the optical absorption associated with the molecular composition of the sample leads to small mechanical actuations emerging at the modulation frequency. This optically-induced sample displacement in turn modulates the probe-sample distance allowing the nonlinear forces of the probe-sample dynamics to encode chemical information. In Figure 1, the probe is driven by piezoelectric (PZT) element exerting two mechanical forces, $F_{p1}(t)$ and $F_{p2}(t)$ at frequencies ω_{p1} and ω_{p2}, respectively. The lowest order coupling synthesizes two modes via sum and difference generation of the excitation frequencies, resulting in a sum mode at $\omega_{p1} + \omega_{p2}$ and a difference mode at $|\omega_{p1} - \omega_{p2}|$. Further, the sample is driven photoacoustically at the synthesized difference mode using an infrared quantum cascade laser (QCL) set at a wavelength where composition-dependent absorption takes place. The QCL energy is delivered to the interrogated region of the sample through an IR-transparent zinc selenide (ZnSe) substrate. The resulting signal S(t) from the microcantilever response via position sensing detection (PSD) forms a chemical map of the cellulose and lignin content of the sample at wavelengths near the 9 to 11 µm "fingerprint" region of the spectra. [Figure 1]

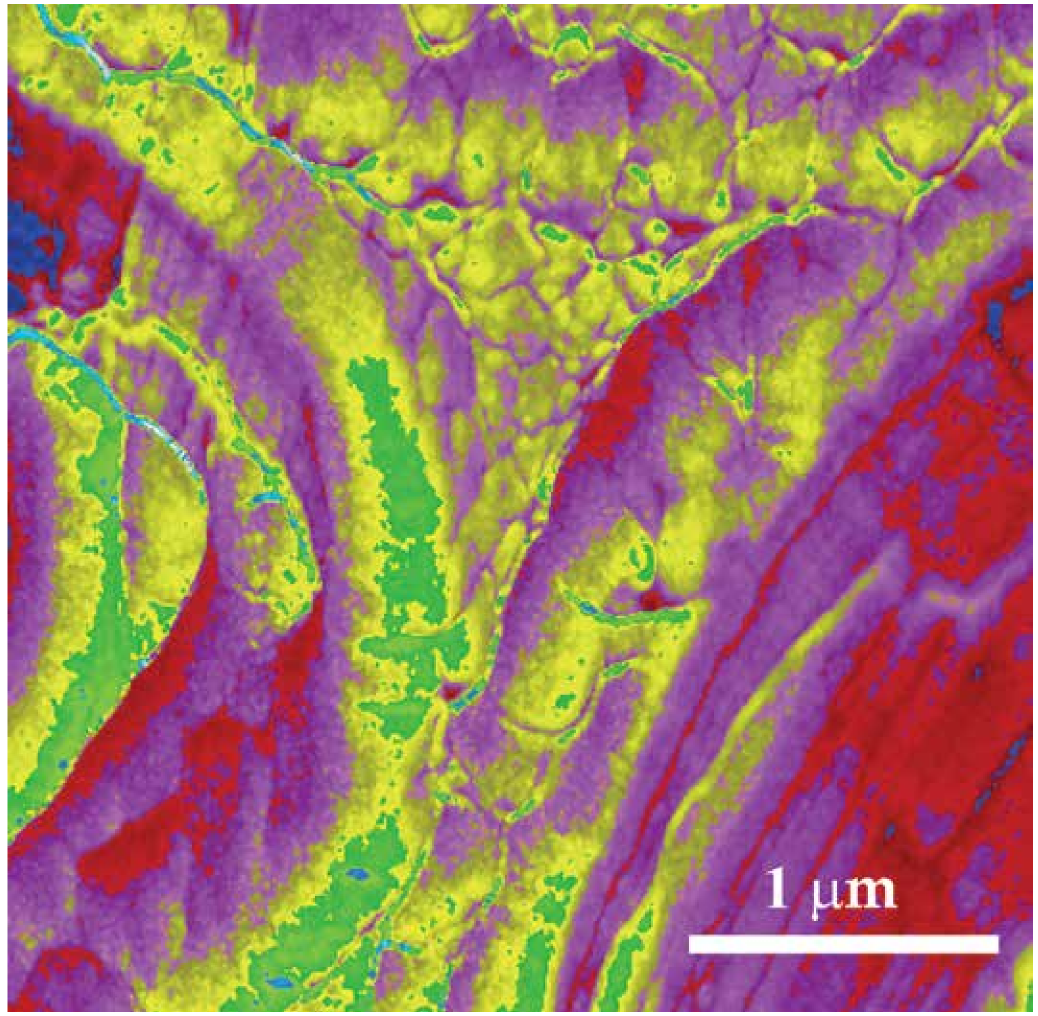

Fig 2: *HPFM image of a cross section of extractive-free Populus revealing nanoscale compositional distinction. The photonic (QCL) excitation is tuned to λ= 1052.63 cm^{-1}. Probe drive frequencies are ω_{p1}= 3.826 MHz and ω_{p2}= 3.800 MHz. An interpretation of the subcellular chemical information of the cell walls can be made for the discontinuous cellulose-rich and lignin-rich regions.*

The specific biomass sample employed in our study is Populus deltoides, which is also known as the eastern cottonwood, a native to North America. Stem cross sections of 20 µm thickness were prepared using various processing stages that are designed to promote biomass conversion to ethanol [Jung *et al.* (2010)]. Fresh samples were left intact after microtoming. Extractive-free samples were the result of the extraction of waxes, fats, resins and other materials that are soluble in neutral solvents not generally considered part of the wood polymer structure. Holopulped samples were created after subsequent oxidative treatment that removes the lignin while preserving the morphology of the sample. Additionally extractive-free samples were subjected to acid hydrolysis to remove hemicellulose. Lignin ("lignum" meaning wood in Latin), a major component in plants and in particular in the plant cell walls, is a hard and rigid material that can be elucidated with HPFM. By characterizing the Populus cross sections with HPFM, nanoscale morphological and chemical tracking of the biomass, as it moves through the processing stages, has revealed the extent of the lignin removal and evidence of increasing amorphousness. [Figure 2]

Conclusions

From the obtained high spatial resolution spectroscopic images, we conclude that the HPFM presents a highly relevant measurement approach for organically complex samples. Naturally, as in any new measurement modality, a maturation process is expected to pave the way for high fidelity data with the ultimate goal of quantitative and high throughput measurement capability.

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