Universität für Bodenkultur Wien
University of Natural Resources
and Applied Life Sciences, Vienna

LEHIGH UNIVERSITY

Final Research Paper

Marshall Plan Scholarship

Research stay 01.05.2019 – 31.07.2019

Title of research project:

Applying advanced chemical force microscopy approaches on functionalized wood

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Host University: Lehigh University
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I. Motivation of the research stay

Wood as a biobased material possesses a unique, hierarchical and porous structure, which gives the possibility to design functionalized materials[1]. Various chemical modification techniques enable to create wood products with innovative characteristics and completely new set of properties. Research groups developed magnetic wood[2], transparent wood[3] or wood water filters[4], to name some examples. Using these advanced techniques, the ligno-cellulosic structure is modified with specifically designed protocols, which allows targeting the functionalization to certain areas in the wood matrix. These nanotechnology approaches request proper and accurate characterization methods to support and confirm these newly developed techniques.

Noy, Vezenov and Lieber[5] described the method of chemical force microscopy (CFM) as a scanning probe microscopy technique, in which a well-defined chemically functionalized tip in the nanometer range is scanning a materials surface. By analyzing the contact mechanism, i.e. adhesion forces of a functionalized tip on the surface of the analyzed sample, it is possible to map a variety of chemical functionalities and surface characteristics. Within the frame of the research stay at Lehigh University, the CFM approach was transferred to the challenging substrate wood to characterize functionalized and native wood materials. During this period, scanning techniques related to CFM were applied at the lab of Dimitri Vezenov on wood structures and ligno-cellulosic substrates with innovative analyzing tools. The goal of this joint project was to conduct nanotechnology characterization methods on high-tech wood materials – a highly needed and novel characterization method in the wood science community.

II. Acknowledgment

I deeply thank Assoc. Prof. Dimitri Vezenov for his time and effort to supervise this joint research approach. Thank you for all the interesting discussions and the ability to perform a variety of experiments. With your help, I could expand my knowledge regarding the analyses of physical and chemical properties appearing on different surfaces.

Thanks to William Leon for the introduction to the applied analyzing tools and his time to answer my questions. Thank you for helping me anytime when I needed your support and the helpful information, which you provided me.

Furthermore, I want to thank Craig Pointer, who helped me anytime when I needed help during my research stay. Thank you for taking your time to support me when I was working in the laboratory.

Additionally, I deeply acknowledge Dr. Xiaoji Xu and Devon Jakob, who helped me to apply Peak force IR measurements on ligno-cellulosic materials. These complex experiments gave us interesting insights into wood materials, which will support our understanding regarding the chemical composition of our substrates.

I deeply thank Dr. Etienne Cabane and his group for providing us with functionalized wood materials to perform the different wood characterization methods.

I want to thank my PhD supervisor Assoc. Prof. Johannes Konnerth, who is supporting me with a lot of effort and who helped me to realize this research stay. With your supervision, my PhD has become one of the most exciting periods in my life due to your positivity and motivation.

Last but not least, I want to thank the Austrian Marshall Plan Foundation for the financial support of this research stay. It was a wonderful opportunity to spend three months at a different university and learn novel research approaches, which was facilitated with this scholarship. In addition, financial support by the Austrian Science Fund FWF, the Lower Austrian Research and Education Society NFB and the Swiss National Science Foundation SNSF is acknowledged.

Content

1 Introduction

The focus of the project, in which the experimental work of this research stay is embedded, is to develop characterization methodologies offered by atomic force microscopy (AFM), which allow analyzing a variety of surface characteristics. In this method a sharp tip with a radius of some nanometers scans the surface of a specimen and by tracing the tip movement with a laser, surface properties are analyzed[6]. With this approach, scientists are able to investigate microstructures, nanoparticles and structural changes of a modification process. Furthermore, AFM enables to conduct force-distance measurements in the pico- to nano-Newton range, which are used to determine the local elastic modulus or adhesion forces between the tip and the materials surface[7]. In 1994 the group of Lieber[8] expanded the AFM possibilities and introduced chemical force microscopy by applying chemically functionalized and well-defined tips, which can be used to determine the interaction between functionalized groups of the tip and the ones of a well-defined surface. The technique can also trace the spatial distribution of functional groups which arise from such a surface. Researchers successfully applied the CFM principle on e.g. modified polymer surfaces[9,10], which are smooth and flat. Due to the heterogeneous and rough wood structure, this nanotechnology method needs adjustments to face the challenges of characterizing wood scaffolds.

With our research approach **we aim at adopting CFM to investigate surface properties** of biobased and thus highly variably native wood, chemically modified wood and ligno-cellulosic materials. At the BOKU Institute of Wood Technology and Renewable Materials micro- and nanoscale characterization methods are applied to investigate novel wood based materials. Our group was already able to successfully characterize the variability in surface polarity of wood[11] or adhesion properties of regenerated lignocellulosic fibres[12] using advanced AFM based techniques. The aim of this PhD project is to analyze functionalized wood substrates with the prior explained CFM principle, since we believe that we can locate introduced polymers in a wood matrix together with analyzing the wood ultrastructure.

The analyzed substrates are amongst others polymerized wood materials on which functionalized polymer chains are covalently bonded to the wood cell wall and inner cell wall surfaces. Various scientists, who are applying such functionalization processes, realized that there is a lack of appropriate analyzing tools and that the current applied characterization methods need to be expanded in terms of combining chemical information with high spatial resolution[13,14]. In contrast to complementary characterization technologies, which are strong for chemical composition characterization (e.g. Raman spectroscopy[15]), CFM is also capable of determining the direct interaction of chemical functional groups together with an outstanding <50 nm spatial resolution. Using the CFM principle, we were already able to visualize the stimuli-responsive behavior of functionalized wood which was modified with a thermo-responsive polymer in situ. By applying CFM on these substrates, we could not only locate polymers in the wooden matrix with high spatial resolution but also prove their predicted function of changing polarity depending on the surrounding temperature (publication in process).

Dimitri Vezenov, a renowned expert in CFM, and his group combine chemistry, physics and engineering to understand and control interactions in chemical systems at small scale. In his research he could prove that scanning probe microscopy is a very powerful tool for the characterization of molecular and functional properties at the nanometer scale[16-18]. By adopting the CFM expertise of Dimitri Vezenov and his group on wood substrates with their inherent high natural variability, we aim at gaining fundamental knowledge and valuable surface

information which will help to better understand the wood ultrastructure and will support wood functionalization processes.
The goal of this research stay is to broaden the knowledge on tip modification techniques and established AFM methods applied at the group of Dimitri Vezenov at Lehigh University on different surfaces. These measurements will therefore increase the mutual understanding of CFM measurements on biobased materials like wood. During this period, **we analyzed ligno-cellulosic fibers, native wood and functionalized wood structures**, which were modified following a certain modification protocol. On these surfaces we applied force titration measurements[18] and Peak force infrared (PFIR) microscopy[19], which are both chemical imaging techniques capable of providing nanoscale resolution. The overall goal was to study the modified wood ultrastructure with a wider range of functionalized tips e.g. –COOH tips and controlled ambient (e.g. liquids with variable pH conditions), and broaden the possibilities to analyze native and modified wood surfaces with CFM. The aim of these measurements was therefore to increase the understanding of CFM measurements on biobased materials like wood and to apply advanced AFM procedures on ligno-cellulosic materials.

2 Research questions

We are convinced that CFM is one key-technique to fill the gap of wood analyzing tools, which are able to directly address the existing as well as the introduced functionalities in a wood matrix. Yet, chemical force microscopy and force titration are novel approaches in wood material science. Force titration is a CFM method in which the adhesion force between chemically modified tips, which terminate in distinct functional groups, and functional groups arising from the samples surface is determined. The adhesion measurements performed in different pH environment can be subsequently used to investigate e.g. the local variability of surface properties like pKa-values, the distribution of acid- as well as base-groups. Force titrations are highly sensitive and demand accurate sample preparation, adjusted scanning settings and proper data evaluation. During the research visit, these analyzing techniques shall be applied on wood substrates. Therefore, the first research question is the following:

> → *How is the experimental design to conduct chemical force microscopy and force titration measurements on wood?*

A complementary method of atomic force microscopy is Peak force infrared (PFIR) microscopy, in which chemical and mechanical maps of the samples surface are simultaneously acquired. The group of Xiaoji Xu at Lehigh University apply this further development of AFM on different substrates in which absorption induced by an infrared laser system will change the movement of the oscillating scanning tip due to photo-thermal expansion and can be converted into a chemical and mechanical signal. With this method, the resolution of chemical mapping was demonstrated to be as low as 10 nm. This method shall give novel insights into the distribution of functional groups and the chemical composition of ligno-cellulosic fibers and wood structures. In addition, modified wood structures shall be analyzed to get an insight into the changes that happen during wood modification procedures. The second research question arises:

> → *How can Peak force infrared microscopy be applied on wood structures and what novel information can be derived from this method?*

3 Materials and Methods

Native wood, functionalized wood or regenerated cellulosic fibers were tested in the experiments.

3.1 Native and functionalized wood structures

Wood is a hierarchical material due to the organization of its components at several size levels. From the annual rings to the molecular structures, a unique organization is visible. In detail, the hierarchical levels in wood derive from the arrangement of the annual rings of a few mm, to wood cells with a diameter of several µm to the cellulose micro fibrils of some nm[20]. Thanks to that unique structure, trees provide remarkable strength properties together with the ability to conduct water with required nutrients. The transport is enabled in the hollow spaces of the wood fibers and e.g. pits, which connect the fibers. The dried wood structure provides therefor porous properties due the arrangement of the hollow wood fibers, which can be used as a matrix to design novel materials.

The wood structure is primarily hydrophilic, which means it is attracted to water and consequently binds water molecules to its structure. That property originates from the abundant hydroxyl groups (-OH) available at the wood surface (Fig. 1). In the present work, we characterized native wood and esterified wood scaffolds. These samples were prepared by the group of our project partner Dr. Etienne Cabane at the ETH Zurich (Wood Materials Science, Stefano-Franscini-Platz 3, CH-8093 Zürich), whose research is focused on the functionalization of wood structures for novel wood applications. Here, the hydroxyl groups of spruce were treated with succinic anhydride towards the design of a wood filter[4] (please find a detailed information and a description of the modification method in the cited publication). Shortly, in that method, the wood samples were dried, then immersed in a solution of solvent (pyridine) with reactant (succinic anhydride) and catalyst (pyridine) for 120 min at 65°C. Then the samples were washed with acetone to remove unreacted chemicals and subsequently they were dried at 65°C until reaching a constant mass. This led to wood modified with an increased amount of carboxylic acid groups (–COOH) inside the wooden matrix to further apply that material as a biosorbent for copper remediation.

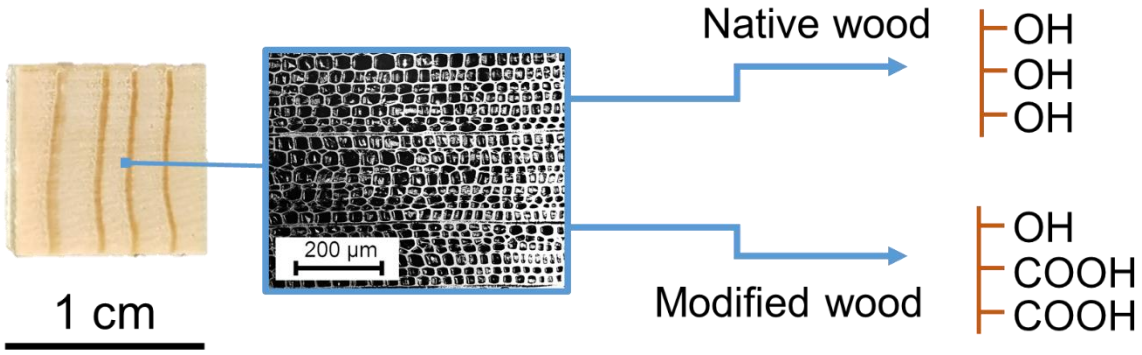

Fig. 1: Native or modified wood samples, in which the porous structure of spruce wood cubes was esterified, were characterized within the research stay.

3.2 Lignocellulosic fibers embedded in PLA

Additional to the wood structures, we analyzed regenerated fibers which we considered as model surfaces since AFM measurements could be hindered by the wood pores. Therefore, method development is facilitated by the utilization of model surfaces. Ioncell-F fibers are produced in a regenerated cellulose fiber process[5] so that lignocellulosic fibers are generated with a variation in the amount of cellulose, hemicellulose and lignin, which represent the main

wood components. These fibers were embedded in a thermoplastic polymer (PLA; polylactic acid) by placing the four types of the fibers aligned in molten PLA[12]. That followed, small cubes were cut with razor blades out of the PLA bock and glued onto metal discs, so that the cross-section of the fibers was pointing upwards (Fig. 2).

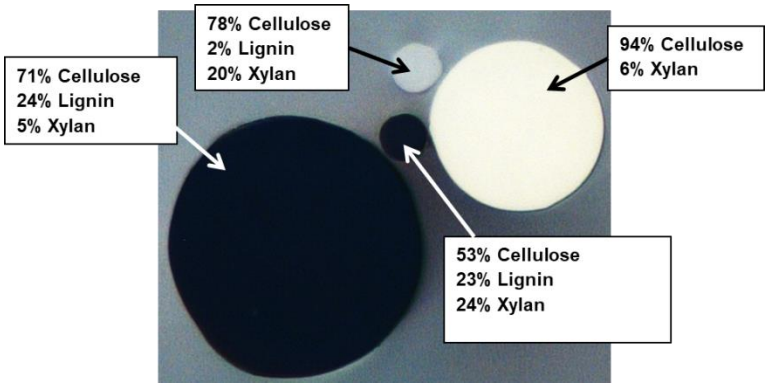

Fig. 2: Four different types of regenerated lignocellulosic fibers were embedded in PLA and were used as model surfaces in AFM measurements.

3.3 Sample preparation

Smooth surfaces are needed to perform any kind of AFM experiments. For this purpose, the samples were cut with an ultramicrotome (Fig. 3, Leica Reichert Ultracut S, Germany) using diamond knives (DiATOME, Switzerland). In detail, small specimens were cut with a razor blade so that an area of 4 x 2 mm^2 can be cut with the device. These small samples were glued (Uhu plus sofortfest, Germany) onto small metal discs and fixed onto the ultramicrotome. The principle of this microtome is the following: the sample is put onto the sample holder and placed in the moving arm of the microtome. The arm is moving with a certain speed and feed and as soon as the sample touches the diamond knife, small sections are removed and a fresh and smooth surface is generated. In case of a force titration measurement, the radial section of a wooden sample or the cross-section of the embedded fibers were cut. In case of Peak force IR measurement, the radial or the transverse section of a wood sample was prepared. Care must be taken, that the microtomed surface stays clean without any contamination which might affect the subsequent measurement. Additionally, the generated surfaces should be controlled with a light microscope to ensure that the cell wall structure is not destroyed or cell wall fragments contaminate the surface.

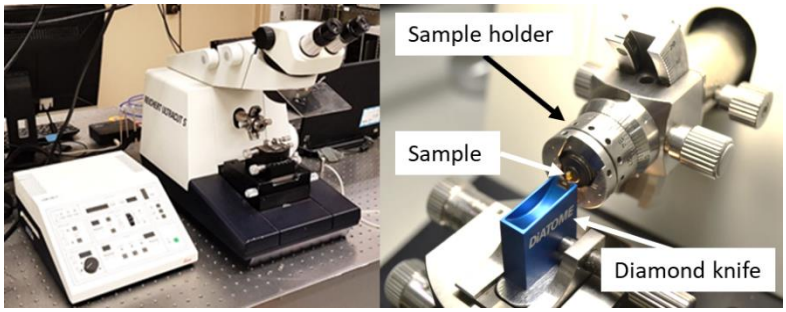

Fig. 3: Smooth surfaces are generated with an ultramicrotome (left image). The specimen is placed on the sample holder and cut with a diamond knife (right image).

3.4 Sample characterization

3.4.1 Atomic force microscope (AFM)

An AFM is based on the ability to scan a specific area of a sample with a sharp tip with a radius typically considerably below 100 nm. The AFM tip is scanning the surface of the sample in a certain pattern. A laser is focused on the backside of the cantilever, which is carrying the tip, and is reflected to a photodiode. By following the movement of the tip, a representation of the topography of the scanned structure is generated. These images are called “false color images”, in which each pixel is representing the position of the tip in the case of e.g. topography maps. In most publications, dark pixels represent low positions of the tip and bright pixels would represent a high position of the tip. If the tip is always in contact with the sample, the measurement mode is called “contact mode”. Another testing possibility of AFM is to perform force-distance measurements on the sample, where the tip touches the sample until a pre-defined force and is then retracted from the surface. Depending on attractive or repulsive forces between the tip and the sample, a certain force is needed to pull the tip away from the surface, which is generally called adhesion force. This force is the characteristic parameter in chemical force microscopy to locally identify different adhesion forces between a chemically functionalized tip and the sample. Thus, with CFM it is possible to track differences in functional groups arising from the surface with specific functionalized tips ending in e.g. –COOH or -CH_3 functionalities.

The measurements at the lab of Dimitri Vezenov were performed with a MFP-3D Bio atomic force microscope (Asylum Research). In this sample-scanning based AFM, the sample is moved in x- and y- direction and the tip moves in z-direction. To perform a measurement, the sample is positioned on the AFM stage and the AFM scan head is placed above the sample, which carries the tip at the bottom (Fig. 4). With a camera system, an area of interest is selected and the tip can be precisely positioned on that area. When measuring a wood substrate, it is important to select an area where the maximum deflection of the tip of some µm is not exceeded during measuring.

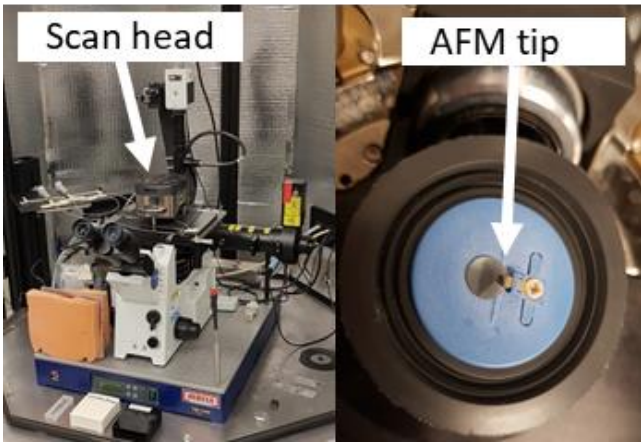

Fig. 4: The atomic force microscope is used to scan the surface of a ligno-cellulosic substrate (left image).The AFM tip is mounted on the bottom side of the scan head (right image).

3.4.2 Tip functionalization

In many AFM measurements tips made out of silicon or silicon nitride are applied for scanning a surface. To add a distinct functionally to the tip, functionalization procedures can be applied.

A common and stable tip modification can be achieved with self-assembled monolayers (SAM). For that, a gold coated tip is placed in e.g. thiol solutions, which on one end form chemical bonds with the gold surface and on the other end, terminate in distinct functional groups. In the present research work, we applied overall gold coated silicon AFM tips (Cont GB-G, Budget Sensors, resonance frequency = 13 kHz, force constant = 0.2 N/m). Two tip modifications were conducted: hydrophilic AFM tips were gained with 11-mercaptoundecanoic acid (95%, Sigma-Aldrich) and hydrophobic tips with 1-dodecanethiol (≥98%, Sigma-Aldrich). The functionalization procedure is as follows: the gold coated tip is cleaned with deionized water and placed in a plasma cleaner (Harrick Plasma). That followed, a thin gold layer is generated on the tip with a sputter coater (Desk II, Denton vacuum LLC). These freshly gold coated tips are then placed in the chosen SAM solution. After 45 min the tips are removed from the SAM solution, cleaned two times in ethanol and one time in water. Finally, they are placed in a buffer solution until they are placed on the AFM scan head to conduct further measurements. Working with AFM tips need high precision since any kind of pollution or damage will influence the final measurements. Complementary methods like scanning electron microscopy can be applied to check the tip geometry after the AFM measurements. The tip functionalization was verified by performing the same functionalization method on glass slides. For this, a glass slide was gold-coated in the sputter coating together with the AFM tips and functionalized with the same SAM solution of each functionalization process. After the functionalization process, a drop of water was placed on the glass slide. A high contact angle between the water drop and the functionalized surface indicates a hydrophobized surface in contrast to a small contact angle, which indicated a hydrophilic, functionalized surface. Chemical force microscopy is an extension of atomic force microscopy, by applying these chemically functionalized and well-defined tips to map chemical variances of the analyzed surfaces. If the CFM measurement is performed with e.g. COOH modified tips in varying pH, changing adhesion forces can be plotted as a function of pH, which is called force titration.

3.4.3 Force titration

At first, the functionalized AFM tip is placed on the tip holder, which is then put onto the AFM scan had and the ultra-microtomed sample is put on the AFM scanning stage in a petri dish. At the beginning of a measurement cycle, the functionalized tip was calibrated. Three parameters need to be calibrated: the deflection sensitivity, the spring constant and the tip radius, which is the only parameter that is determined after the measurement. After determining the deflection sensitivity and the spring constant, a height image which represents the topography of the sample is generated in contact mode to find an optimal position of a wood cell wall in air as environment. If the wood cell wall is showing sufficiently smooth surface properties, the measurements in fluid environment can be executed. For that, the tip is withdrawn from the surface, the petri dish is filled up with the first buffer and the wood sample is allowed to swell for 45 min. Subsequently the tip is placed on the prior selected wood cell wall area until a pre-defined set-point. Due to the swelling properties of wood, it could be difficult to follow the preselected wood cell wall area when changing the buffer solution. Additionally, care must be taken when readjusting the tip and laser position since too high forces could damage the tip. An adhesion force map is generated, in which force-distance measurements are performed in a certain pattern. After that map is generated, adhesion force maps are acquired in the next buffer at the same measuring position. In total, adhesion force maps were generated in the following phosphate buffers: pH 2, pH 4, pH 6, pH 7, pH 8, pH 10, pH 12 and the measurement at the first pH-value was repeated to see if the measured

adhesion forces are reproducible. After the force titration cycle was finished, a contact mode image was taken to track structural changes in the wood cell wall. The adhesion force is calculated out of the force distance curve (Fig. 5) in Igor Pro (WaveMetrics) and certain areas on the wood cell wall were masked to calculate an average adhesion value of the masked area. The adhesion force is defined as the difference between the maximum force to pull the tip away from the surface and the baseline, when the tip is not in contact with the wood surface. A force titration refers to adhesion measurements which are made as a function of pH[18]. At a sharp decrease or increase of the adhesion forces, the pKa value of the analyzed surface can be estimated. Therefore, we should be able to determine the pKa value of a native or unmodified wood cell wall with the force titration principle. Care must be taken that the measurement settings stay constant during a whole measurement cycle to gain comparable adhesion values.

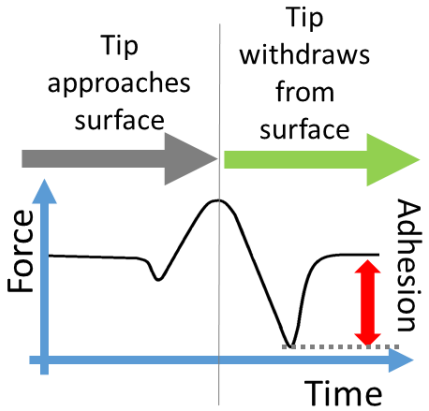

Fig. 5: The adhesion force is calculated from the force curve depending on e.g. attractive force between the tip apex and the chemical property of the sample.

3.4.4 Peak force infrared (PFIR) microscopy

In Peak force infrared (PFIR) microscopy an atomic force microscope is coupled with an infrared laser source. This imaging technique is applied by the group of Dr. Xu at Lehigh University[21] in collaboration with an industrial partner. In principle, Peak Force QNM (Quantitative NanoMechanics) in an advanced Tapping Mode™, which allows quantitative measurements of nano-scale material properties (e.g. modulus, adhesion, deformation, indentation) simultaneously. In this mode the tip performs force–distance maps on the fly, whereby the topographical and mechanical maps are generated simultaneously.

An IR laser is focused between the AFM tip (Bruker, Platinum tip, k = 39 N/m) and the surface of the sample, which will induce photo-thermal expansion. That expansion is recognized by the deflection of the AFM tip and depends on the chemical composition of the sample. Since the infrared laser pulses and illuminates the surface under the AFM tip every other cycle, one can subtract the vertical deflection of the tip from the movement of the tip with and without the laser-induced deflection (Fig. 6). That signal is processed and will give information on the infrared absorption. In one measurement mode, the infrared frequency is set to a certain value and the laser -induced responses are visualized when the tip maps the surface. In a different mode, the tip measures on one position the thermal expansion of that single spot and the frequency of the infrared laser is varied. Therefore, topographical, mechanical and chemical maps can be measured simultaneously with Peak force infrared microscopy. The method could

be successfully applied on block-copolymers[21], aerosol particles[22] and oil shales[23] to track chemical variation of the surface with high spatial resolution.

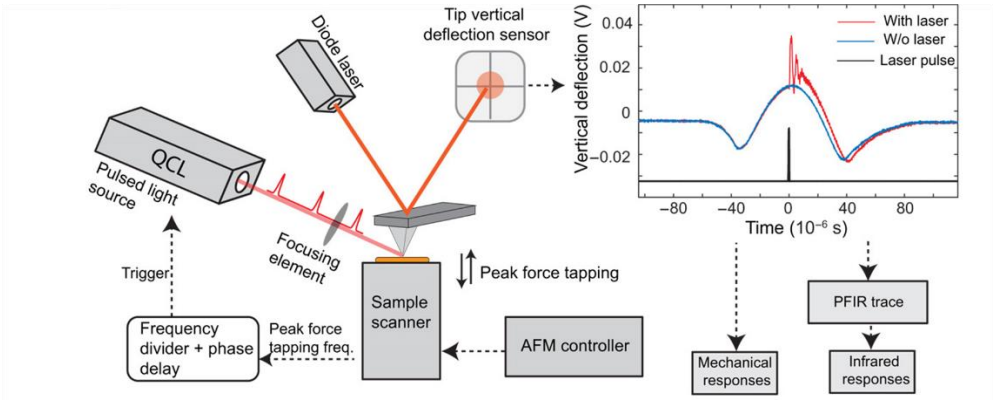

Fig. 6: The principle of Peak force infrared microscopy, which is applied by the group of Dr. Xu at Lehigh University (image taken from Wang et al. 2017).

4 Results

4.1 Adhesion force mapping on unmodified spruce wood in air

The goals of this experiment were to visualize and check contrasts in adhesion forces with the applied tip functionalization chemistry in air and to get familiar with the atomic force microscope measurement settings. The measurement position was chosen so that the S2 cell wall layer together with the middle lamella is scanned. The so-called S2 cell wall is the thickest cell wall layer of a wood cell whereas the middle lamella acts as a connecter of neighboring cell walls. In the first measurement, a hydrophobic tip, which was functionalized with 1-dodecanethiol, was scanned over an ultra-microtomed, unmodified radial section of a cell wall in contact mode. A height image revealed the structural properties of the cell wall (Fig. 7). The bright area in the left of the height image is a section of the middle lamella and the majority of the scanned surface represents the S2 cell wall. An adhesion force map was performed on the same position. Bright pixels on the left area of the adhesion map are areas in which the tip shows higher affinity towards the middle lamella and dark, grey areas are areas in which the tip shows lower adhesion forces towards the S2 cell wall. The average adhesion force of the middle lamella of 200.5 nN was calculated in IgorPro (standard deviation 29.4 nN) and is higher than the average adhesion force in the S2 cell wall (116.2 nN, standard deviation 34.4 nN).

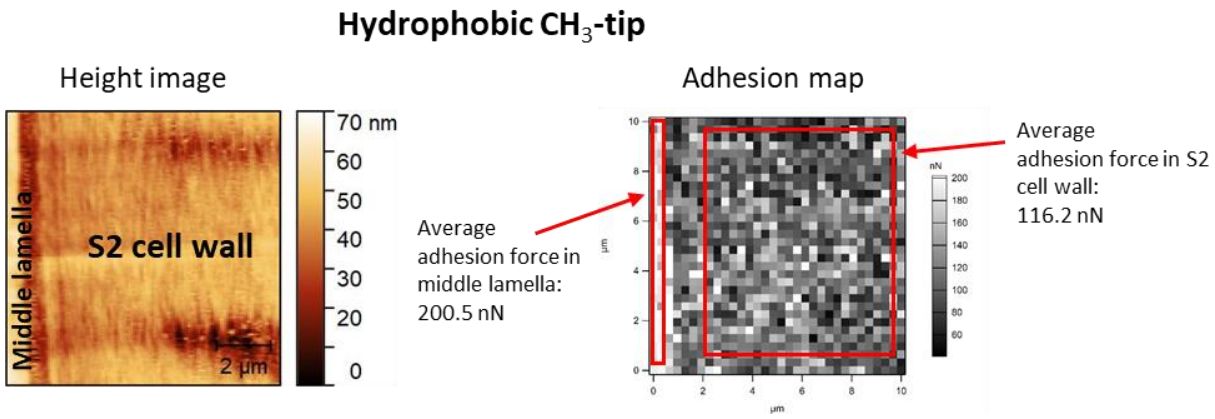

Fig. 7: Height image (measured in contact mode) and corresponding adhesion map of a native wood cell wall imaged with a hydrophobic atomic force microscopy tip.

In the next measurement, a tip with hydrophilic properties was applied to firstly scan a native wood cell wall in radial section and secondly to map adhesion forces on the same position. The height image revealed the structural properties of the scanned wood surface similar as in the previous measurement and clear contrasts between the middle lamella and the S2 cell wall are distinguishable (Fig. 8). The average adhesion force of the middle lamella was calculated out of the masked area of the adhesion force maps (53.2 nN, standard deviation 10.21 nN) as well as the average adhesion force of the masked area corresponding to the S2 cell wall (53.3 nN, standard deviation 7.1 nN). Contrary to the adhesion maps in Fig. 7, the adhesion forces between the wood cell wall and the middle lamella do not differ which means that the hydrophilic tip shows hardly any contrasts regarding the S2 cell wall and the middle lamella. Comparing Fig. 7 and Fig. 8, higher adhesion could be achieved with hydrophobic tips, which also showed higher contrasts between the two masked areas (middle lamella and S2 cell wall).

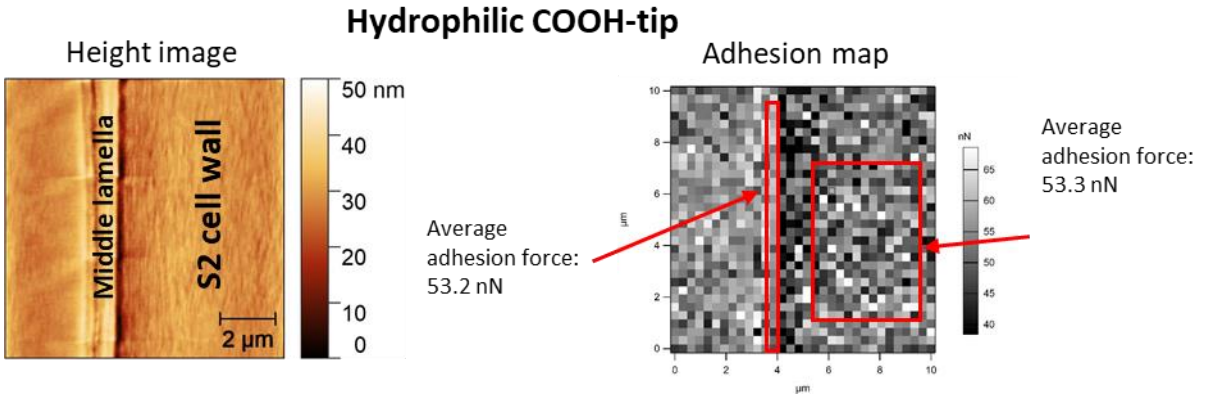

Fig. 8: Height image (measured in contact mode) and corresponding adhesion map of a native wood cell wall imaged with a hydrophilic atomic force microscopy tip.

4.2 Adhesion force mapping in aqueous environment on wood scaffolds

The challenge of performing AFM measurements on wood structures in aqueous environment is the swelling of the wood matrix. The swelling leads to a shift in the wood structure, which hinders the constant mapping of the same scanning position. Therefore, a set-up was

developed, in which the tip stays nearly constantly in contact with the surface so that the area of interest can be tracked. The height of a standard AFM fluid cell was increased so that the comparatively high wood samples were completely covered by the buffer solutions. In addition, tubes were connected to the fluid cell, so that the buffers can be exchanged without moving the tip or changing the scanning position (Fig 9). A further challenge in tracking the same measuring position was the difficulty to follow the wood cell wall by the camera top view in liquids. A focused picture of the surface of the sample could be acquired in air, so that the AFM tip could be precisely positioned on the wood cell wall. That camera view gets blurry when the wood sample was floated with a buffer solution or the buffer solution was changed.

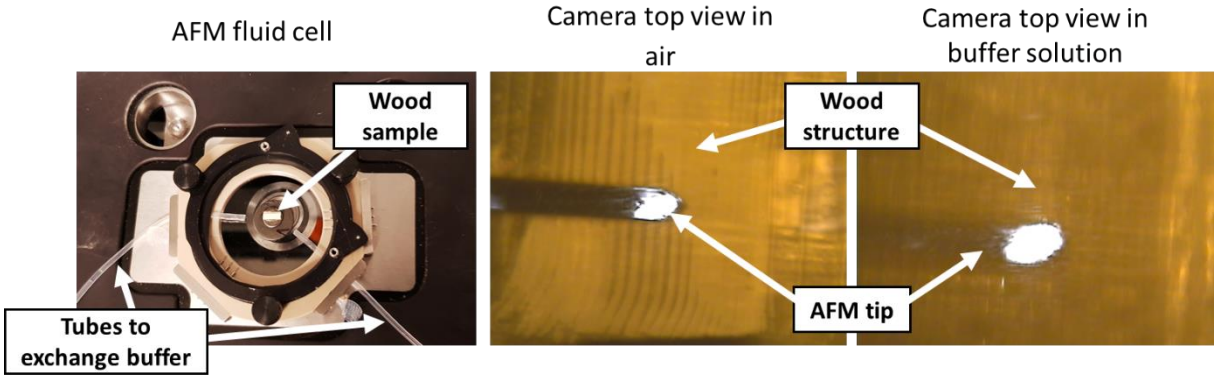

Fig. 9: Set-up of the AFM fluid cell (left image) and camera views of tip on same position on the wood surface in air (middle image) or in buffer solution (right image).

Therefore, we developed a method to follow the same measuring position when the wood structure was swollen. The first step of that procedure was to choose an area of interest in which the neighboring wood cell walls are flat and did not possess large lumina. This prevented tip damage if there was a big shift of the position of the area of interest. Additionally, after filling up the fluid cell with a buffer solution, care was taken when the wheel of the AFM scanner was moved to withdraw the tip from the surface. If too much force was applied to handle the wheel, the tip position was shifted up to several µm. It was important to increase the gap between the tip and the wood surface, so that there was enough space to allow swelling of the structure, which could result in crashing the fragile tip. In addition, it was important to check that the expansion due to the swelling was constant. Therefore, before performing adhesion maps, a low resolution image revealed if the swelling was constant and how far the area of interest was shifted. If that was achieved, force maps could be acquired without moving the tip by changing the buffer solutions with syringes that are connected with the fluid cell by tubes. That method allowed to perform smooth adhesion mapping of the wood cell wall in aqueous environments.

In general, we observed different behavior in the force measurements on the wood surfaces (Fig. 10). As described above, the adhesion force is defined as the difference between the maximum force which is required to pull the tip away from the surface and the baseline, when the tip is not in contact with the wood surface. Low or no adhesion was observed, when the red line (movement of the tip towards the surface) and the blue line (tip movement away from the surface) were nearly completely overlapping. That behavior explained that no or hardly any attractive forces between the AFM tip and the wood surface were present (Fig. 10a). If there is a sharp pull-off of the tip after the maximum adhesion was reached, a clear or simple adhesion behavior is recorded (Fig. 10b). Two more complex adhesion phenomena are molecular peeling and molecular stretching. At molecular peeling, a molecular chain is assumed to be “grabbed” by the tip due to attractive forces and was peeled off the surface until a certain force, when the chain snaps back to the original baseline position (Fig. 10c). At

molecular stretching, the tip also pulls a molecular chain from the surface but unfolds a molecular structure. In between that unfolding step, stretching of the molecule led to several high adhesion forces (Fig. 10d). All of these four adhesion phenomena were recorded on native and unmodified wood scaffolds.

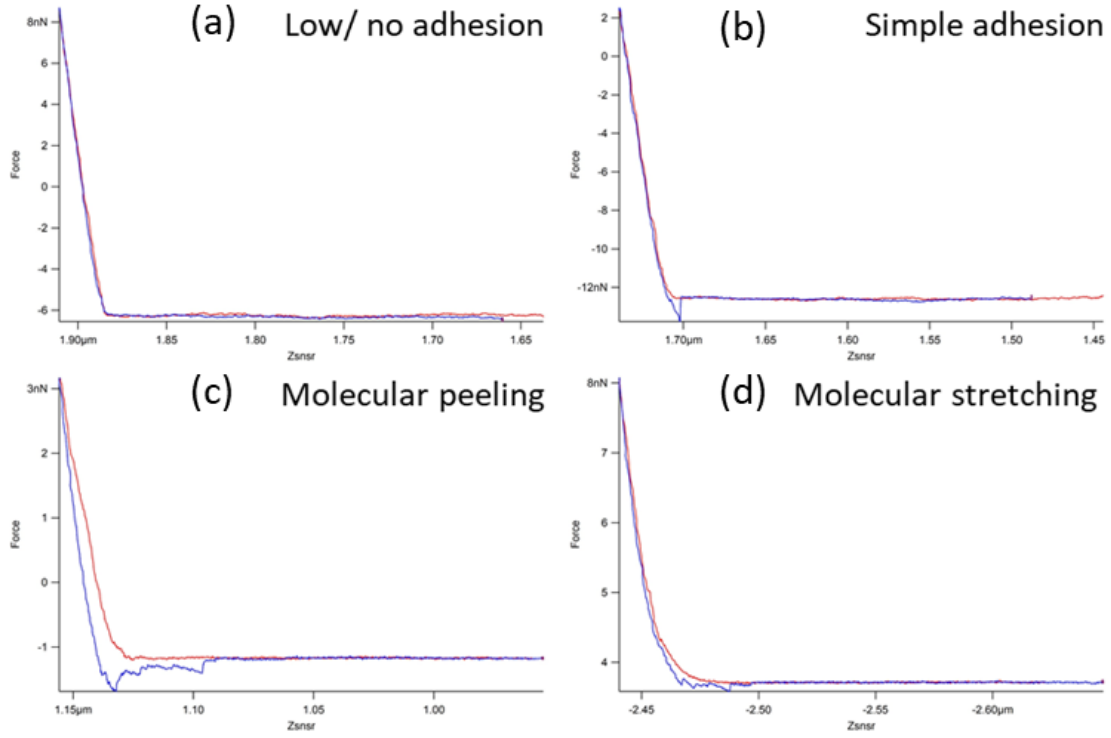

Fig. 10: Force-distance curves analyses revealed different adhesion phenomena on wood structures, when the measurements were acquired in aqueous environment. The red line shows the movement of the tip towards the surface and the blue line represents the tip movement away from the surface.

4.3 Adhesion force mapping on unmodified and esterified spruce wood in varying solution pH

In a further experiment, force maps on native and esterified wood structures, which refers to "Wood-COOH"), were acquired with a -COOH modified tip in varying pH. In first trials, large areas on the wood substrates were scanned (up to 50 µm) with high resolution to also visualize variances between the different wood cell wall layers (as seen in Fig. 7 and Fig. 8). This led to long measurements, in which probably too much tip wear took place and the tips did not endure the whole cyclic force titration measurements. Thus, the scan size was decreased to 10 µm to reduce the probability of breaking the AFM tip and only the S2 cell wall area of a radial section was scanned. With that settings, full force titration cycles could be performed and the average adhesion of the S2 cell wall areas was plotted as a function of pH (Fig. 11). At the force titration measurements of "unmodified spruce wood Nr.1", "COOH-wood Nr.1" and "COOH-wood Nr.3" the pH was increased from pH 2 to pH 12 by changing the buffer solution and at the measurements of Nr.2. of unmodified wood and Nr.2 of COOH-wood the pH was decreased from pH 12 to pH 2. That variation should decrease the effect of the duration of the measurement on the tip performance. Note that all gained adhesion forces between a COOH-modified tip and the swollen native and also modified wood structures were really low (below 1 nN). Changing the scanning settings could increase the adhesion forces only slightly.
The average adhesion force, which was acquired on unmodified wood samples (red lines Fig. 11), was showing a pH dependent behavior and was increasing with increasing pH

independent of starting the force titration at pH 2 or pH 12. In that cycles, a plateau of roughly 160 pN from pH 6 to pH 8 was present before higher adhesion forces of approx. 400 pN were obtained.
The average adhesion forces acquired on COOH-wood surfaces did not show the same trend of the unmodified wood scaffolds (blue lines Fig. 11). Here, a random variation of the average adhesion forces at the S2 cell wall layer were obtained.

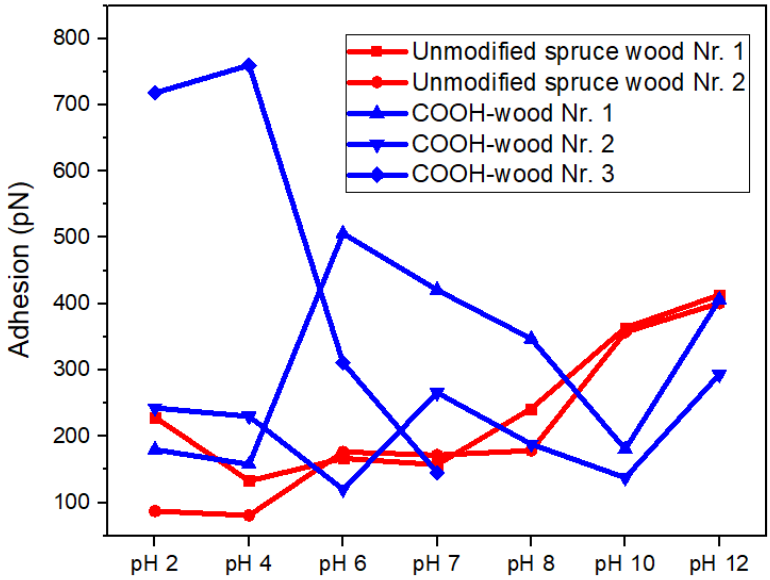

Fig. 11: Adhesion changes at the S2 cell wall of unmodified (red line) and esterified (blue line) wood scaffolds obtained with a hydrophilic tip (-COOH modified tip).

One of the force titration rows (COOH-wood Nr.3, blue line in Fig. 11) could not be continued due to unknown influences during the measurement. A height image of the scanning area at pH 2 and pH 8 revealed that changes occurred due to pollutions at the tip, changes at the wood surface, disturbing particles in the buffer solution or damage/ breakage of the tip (Fig. 12). That behavior occurred on several wood samples so that these measurements could not be finished until all pH maps were acquired.

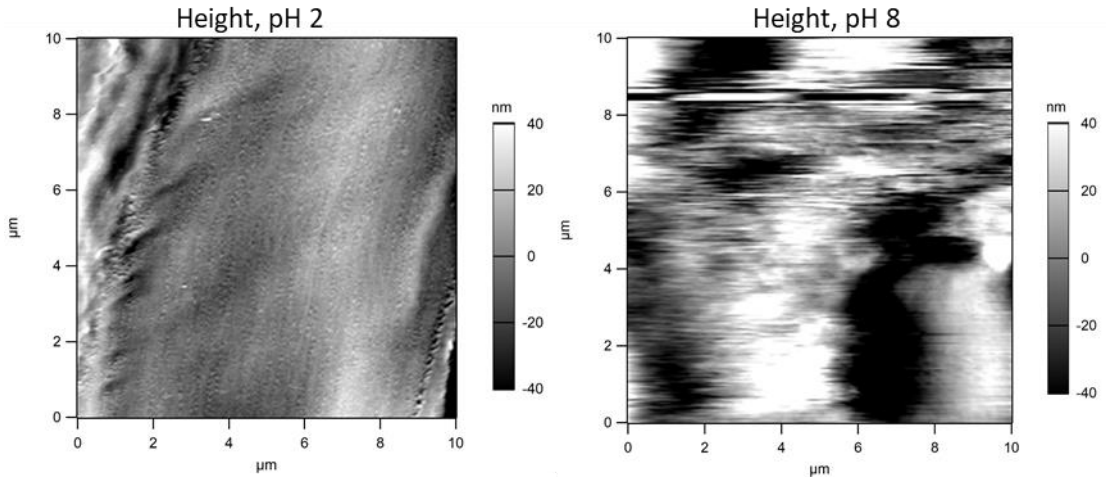

Fig. 12: Changes in the height image acquired on a wood surface at two different pH values. Changes in the buffer solution, at the AFM tip or at the surface of the sample lead to difficulties in conducting long-term measurements.

In addition to the previous measurements, force titration measurements were obtained with hydrophobic tips (-CH_3 tips) at varying pH since we expect higher adhesion forces with these tips[24]. The measurements were as well conducted on an esterified wood surface (COOH-wood). The adhesion force between the hydrophobic tip and the modified wood scaffold showed low or hardly any adhesion forces (between 10 nN and 30 nN, Fig. 13) and it is in a way impossible to see a trend in the varying adhesion forces. Anyhow, the average adhesion forces showed a small trend towards higher adhesion forces at lower pH. Compared to the mapping with COOH-tips (Fig. 11), much lower forces were obtained with hydrophobic tips. Again, it was really challenging to follow the measuring position, so that only one complete force titration cycle could be obtained.

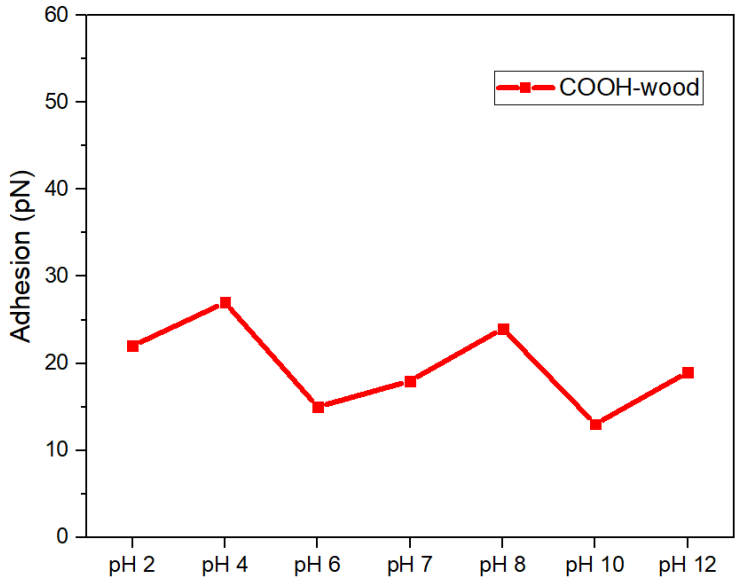

Fig. 13: Adhesion changes at the S2 cell wall of an esterified wood scaffold obtained with a hydrophobic tip (CH3-tip).

4.4 Adhesion force mapping on ligno –cellulosic fibers in varying solution pH

The ultra–microtomed cross-section of regenerated fibers embedded in PLA were characterized with a hydrophilic COOH-tip. Since these wood fibers should show less swelling properties, lower roughness influences and possess a more homogenous surface characteristic, we applied the force titration principle on these surfaces. A full force titration cycle could be performed and the average adhesion of each fiber was plotted as a function of pH (Fig. 14). Adhesion forces up to 1 nN could be acquired and the average adhesion force was increasing with increasing pH regarding the ligno-cellulosic fibers. The PLA polymer did not show a systematic trend or behavior similar to the ligno-cellulosic surfaces when analyzing the dependency on the varying pH.

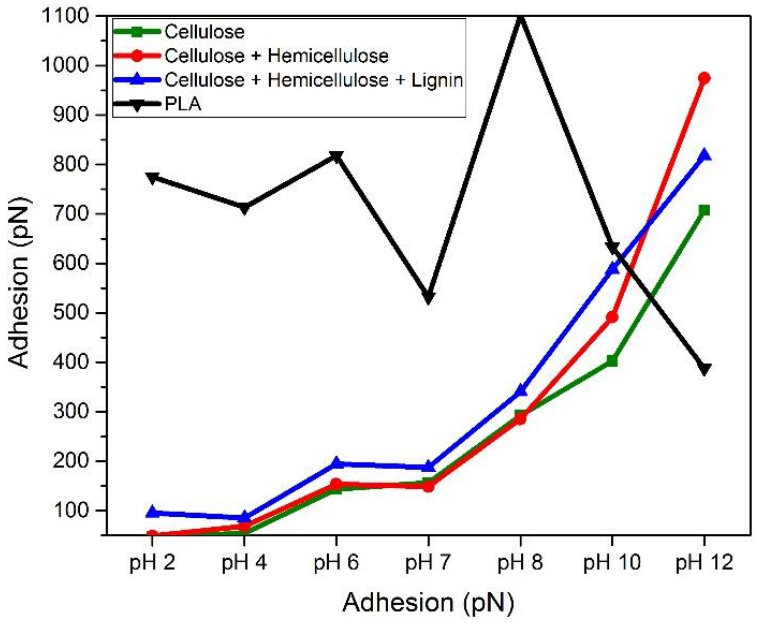

Fig. 14: Adhesion changes obtained at the cross-section of lignocellulosic fibers embedded in PLA acquired with a hydrophilic tip (-COOH modified tip).

At that sample disturbances occurred because the fiber with the highest amount of lignin detached from the PLA matrix and the tip always “get caught” at the interface between that fiber and PLA (Fig. 15). Therefore, in that case the measurement could not be continued and the measurements at that the fiber were skipped.

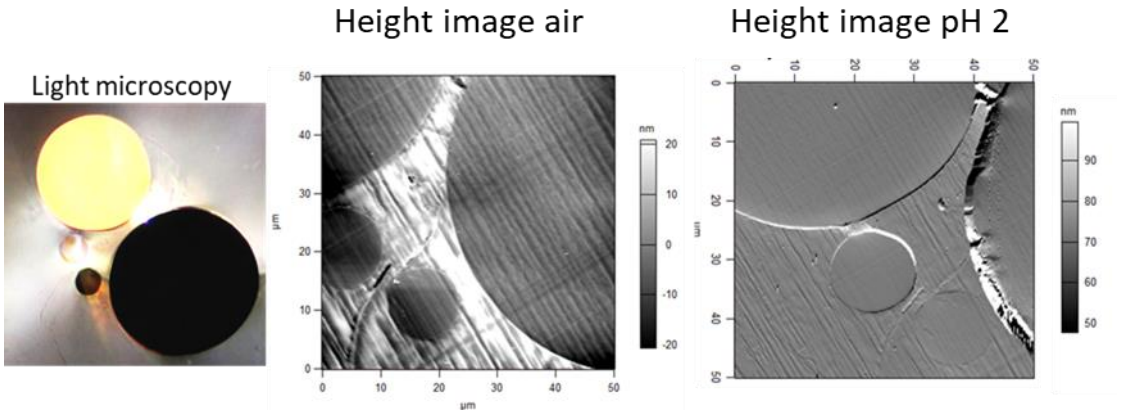

Fig. 15: Structural changes at the regenerated fibers when conducting measurements in different pH values.

4.5 Peak Force IR

The goal of these measurements was to locate functional groups at an ultra-microtomed wood cell wall layer by Peak force IR on native spruce wood structures. For that a wood cell wall was chosen, which provided a large microtomed S2 cell wall layer without big height steps between the cell wall and the lumen area (Fig. 16). Quick AFM height images were taken at the selected wood call wall area before the IR laser was aligned to verify that the tip position scans the desired wood cell wall parts.

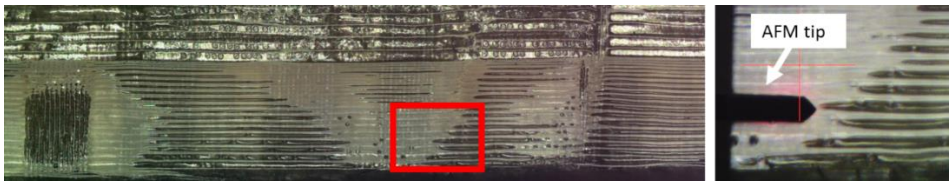

Fig. 16: Measuring position of a Peak force IR measurement at the radial section of native spruce wood. The scanning area was chosen so that the cell wall provides smooth and large cell wall areas.

In the next step, the IR laser was aligned between the AFM tip and the wood cell wall, which led to photo-thermal expansion depending on the selected IR laser wavelength. Care was taken, that the pulsed IR laser was well aligned with the Peak force tapping cycles. Depending on the characteristic peaks of a Fourier transform infrared spectrum obtained with conventional IR spectroscopes, the laser was tuned to different wavenumbers to map the surface of the S2 cell wall. It has to be noticed, that chemical maps could only be compared quantitatively, if the alignment of the IR laser was not changed. Since this technique is so sensitive, a change in the laser alignment will lead to different intensities in the deflection of the cantilever. Peak force IR maps gained on unmodified spruce wood revealed the distribution of different absorption intensities at the transition from the wood cell wall (Fig. 17A, left area in topography image) to the middle lamella (Fig. 17A right area in topography image). If a position shows high thermal expansion, bright pixels in the chemical maps will be displayed and reveal that the functional groups at that position absorb more laser-induced energy at the specific wavenumber. The laser was tuned to 1110 cm^{-1} to track bands corresponding to cellulosic features[25]. The cell wall area showed higher absorption than the area of the middle lamella (Fig. 17B). Tuning the laser to 1512 cm^{-1} revealed the distribution of lignin (Fig. 17C). Overall higher absorption intensities were obtained in these scans compared to mapping at 1110 cm^{-1}. Slightly higher absorption was measured at the middle lamella area. Analyzing profile lines of these absorption signal revealed, that the spatial resolution of these maps is below 15 nm! On the same area, also adhesion maps of the tip on the native wood surface were analyzed (Fig. 17E) and revealed insights also into adhesion force differences between the AFM tip and the wood surface. Additionally, nanoscale infrared spectra were acquired at two different positions with PFIR and revealed slight differences between a spectrum taken from the cell wall area (Fig. 17D, red line) and the middle lamella (Fig. 17D, blue line).

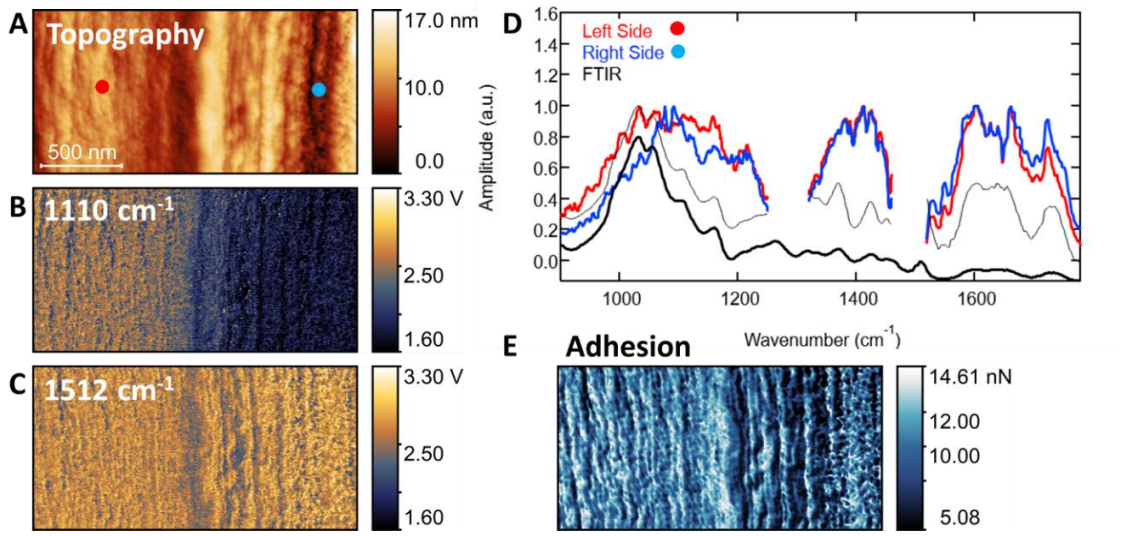

Fig. 17: Height image (A) of selected wood cell wall area and corresponding chemical maps acquired at different wavenumbers (B and C). Spectral data acquired at two different position (D, positions is indicated with red/blue circle) and adhesion map of the same area (E).

5 Discussion

With the applied methods chemical nanoscale characterizations were executed: force titration measurements and Peak force IR. The following observations were made:

Atomic force microscopy measurements in air provided insights into chemical variances of the scanned surfaces by analyzing adhesion forces with functionalized tips. Since wood binds water to its structure depending on the relative humidity of the surrounding environment, capillary forces influenced the measurements. To eliminate these influences, measurements were obtained in specific liquid solutions.

Force titrations measurements were conducted in different phosphate buffer solutions. The swelling of the wood structures in an aqueous environment complicated the AFM measurements. Frequent readjustments of the tip and realignment of the laser were needed due to shifts of the measuring position caused by the swelling property. For these readjustments, the AFM scanner was touched which changed the position of the tip so that several force titration measurements could not be finished. This led to changes of some micrometers which sometimes result in landing on a different position and needed much effort to find the previous scanning position.

Another property of wood, which has to be considered, is the natural heterogeneity regarding the chemical composition and structural characteristics. Every wood cell wall possesses different cell wall thicknesses or slightly different angles of the wood components. Additionally, the chemical composition varies within the cell wall. These effects have influences on measured forces between the AFM tip and might explain the variances in the average adhesion forces acquired with force titration.

Peak force IR mapping revealed chemical properties on the nanometer scale and nanoscale infrared spectra could be acquired on preselected positions. This is a completely novel characterization technique in the wood materials community.

6 Conclusion

For the development and improvement of novel wood based materials, proper characterization methods are required. To use the full potential of wood materials and to increase the current wood applications, nanoscale analyzation methods are needed. Within the research stay two powerful nanoscale chemical analyzing techniques were applied on ligno-cellulosic structures: force titration and Peak force IR. The first task was therefore to optimize the experimental design to conduct chemical force microscopy and force titration measurements on wood. Secondly, Peak force infrared microscopy was applied on wood structures to aim at gaining novel information regarding chemical surface properties. To perform force titration measurements, we developed an experimental set-up for a whole chemical force titration cycle. From AFM tip functionalization, to sample preparation, to optimizing the measurement settings and data analyses – all these procedures were optimized during the research stay. The standard measurement set-ups needed to be adapted to meet the requirements to perform the AFM measurements on challenging wood surfaces. The measurement set-up developed at Lehigh University will be applied in future research activities to improve the understanding of the ligno-cellulosic materials, which are analyzed at the Institute of Wood Technology and

Renewable Materials. Additional, high spatial resolution chemical mapping with Peak force IR revealed novel insights into wood scaffolds. The spatial resolution of chemical maps was found to be <15 nm, which turns Peak force IR into an outstanding characterization technique. Yet, this measuring device is not available commercially, which is why it was a great opportunity to get acquainted with this novel method. In the research visit from 01.05.2019 until the 31.07.2019 final surface preparation needed for CFM was performed at Lehigh University, together with the various characterization approaches including advanced AFM and Peak force IR measurements. Jointly evaluating the gained results was as well started at Lehigh University. Both techniques, force titration and Peak force IR were not applied on wood substrates to our knowledge.

7 General impression of research stay

Working at Lehigh University was an enrichment and a valuable experience in our research project. It was a research exchange in many ways: firstly, new methods to conduct tip functionalization processes, calibration procedures, experimental design and data evaluation were gained and will be further conducted at the facilities at the Institute of Wood Technology and Renewable Materials. Furthermore, a bridge was built between the groups of Dimitri Vezenov, Xiaoji Xu and our institute for further discussions and knowledge exchange. Additionally, we transferred our knowledge on wood functionalization and sample preparation regarding ligno-cellulosic substrates to Lehigh University and showed to different research groups the potential of wood based materials. We are convinced that the gained knowledge and expertise will further optimize the quality and outcome of the applied experiments at our research institute. The research stay represents a perfect example of the importance and possible progress made during a short-term scientific missions to transfer or rather interchange scientific knowledge and information in a non-competing scientific field. It was a wonderful opportunity to learn from a different research lab novel experimental approaches and get as well insights into the differences in research attempts and PhD structures depending on the university and country.

III. Table of figures

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