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# Automated Handling of Bio-Nanowires for Nanopackaging

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***Abstract*—The integration of biomaterials into micro/nano-sensors or micro/nano-systems is expected to improve the properties of such systems or even lead to the development of novel innovative systems. A key problem to be solved beforehand is the development and realization of proper preparation, handling and manipulation methods with respect to an industrial usage. To enable such a usage, the methods have to be automatable, robust to environmental changes as well as feasible in a scanning electron microscope (SEM). According to these points, the target of the presented efforts is to develop these methods for a future design of nanoelectronic parts and to solve packaging problems at the nanoscale. As a consequence, the paper presents a novel concept for the usage of biomaterials, such as DNA and wood fibers/fibrils, for the packaging at the nanoscale. Novel methods for the DNA-handling with an atomic force microscope (AFM) at dry conditions, which can also be used in the vacuum chamber of a SEM will be presented as well as wood fibers/fibrils manipulation methods in the SEM.**

## I. Introduction

In this paper, current research work on automated handling of bio-nanowires for nanopackaging is presented. A main research focus of the authors divisions tackles the manipulation and handling of biomaterials like DNA or cellulose fibers and fibrils. For this, we develop methods to combine commonly used self-assembling strategies with automated methods based on the use of an atomic force microscope (AFM) or based on the use of microgrippers. In this context, the paper presents a concept to solve problems for the electronic packaging at the nanoscale, methods to handle immobilized DNA at dry conditions as well as cellulose fibers and fibrils.

The importance of nanoelectronics for the future is well-recognized. By now, it is subject of various industrial policy roadmaps. Next-generation nanoelectronic technologies, (e.g. single-electron transistors, molecular electronics, etc.) are sensitive to dimensional change. Therefore, an appropriate packaging is essential to the success or failure of these technologies. Packaging strategies must therefore be developed parallel to the basic nanoelectronics device technologies in order to make informed decisions on their commercial viabilities [1].

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To overcome problems during the miniaturization in microelectronics and enable nanoelectronics, the use of objects like carbon nanotubes (CNTs) or biomolecules such as DNA is necessary for future nanoelectronics. Today, it is well known that nanowires produced via a selective metallization of DNA are essential for future nanoelectronic devices [2-9]. Based on their properties, DNA can be considered as a basic building block for nanoelectronic devices [10] as well as for nanoscale bonding wires. Capitalizing on the geometrical properties of DNA nanoscopic wires with diameters below 10 nm are possible [2, 6-9]. Also, an integration of DNA into specific predefined sites of microstructured or nanostructured electronic circuits is possible due to the possibility of functionalizing the ends of the DNA strands with various linker molecules. Furthermore, complex structures made of DNA can be fabricated [6, 11] and metalized at a later stage [12]. To design nanoelectric circuits by using DNA, various approaches have to be evaluated. However, the handling of DNA has been an area of intense research in recent years and many approaches were shown or demonstrated. So optical tweezers, magnetic tweezers, microfluidic handling systems, laser-based dissection methods, dielectrophoresis, nanotweezers and also the AFM for lithographic structuring of substrates are used for the handling and manipulation of DNA [13, 14, 15] up to now. These methods can all be used to solve special research problems, but the usage of DNA as a material for industrial processing of electronic circuits or material for nanobonding is not possible, because of the complexity of the necessary setups. An automation of most of these setups cannot be achieved or isn't even feasible. Furthermore, to build nanostructures out of DNA, self-assembling technologies can be used [15, 16]. These approaches are very promising, but it needs also to be taken into account that current methods and experiments cannot be used for an automation of the processes, which is a key value for industrial use. So our main goal is to enable the usage of DNA as a conductor not only for research use but also and mainly for a future industrial use.

Compared to DNA, other organic materials, such as micro fibrils from wood fibers, are recently become interesting for international research [17-19]. However, first successful metallization experiments of structures made from organic cellulose [20] make them a possible successor for the pure metallic bonds in micro- and nano-system technology, which are about 20 µm in diameter or bigger. These cellulose-based building blocks can be obtained from very different sources: Certain bacteria produce strands of cellulose. Other

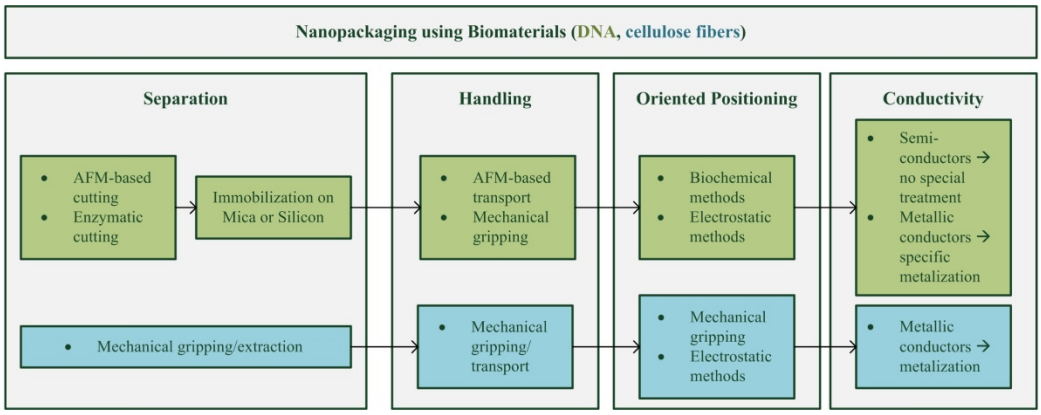


Fig 1. Working scenarios for the use of biomaterials (DNA – green, upper row; cellulose fibers – blue, lower row) as bonding wires for nanopackaging.

sources are for example algae or wood fibers [20, 21]. In textile industry, technically produced cellulose fibers are available. The size ranges from about 20 nm for bacterial cellulose up to several hundreds of nanometers for bundles of fibrils of wood fibers. Industrial fibers are some hundreds of µm in size. All sources are easily accessible.

In the following sections, we introduce a first concept for packaging at the nanoscale by using DNA and wood fibrils. Methods for preparation, handling and manipulation of biomaterials will be described in order to solve packaging problems, being characteristic for the nanoscale, enabling the usage of automated methods. Section II shows our concept of electronic packaging at the nanoscale by using biomaterials. The first results for preparation, handling and manipulation of DNA at dry ambient condition are presented in Section III. In Section IV first experiments on preparation, handling and manipulation of cellulose fibers are presented. Finally, the paper is concluded in Section V, including a short outlook.

## II. Towards Nanopackaging Using Biomaterials

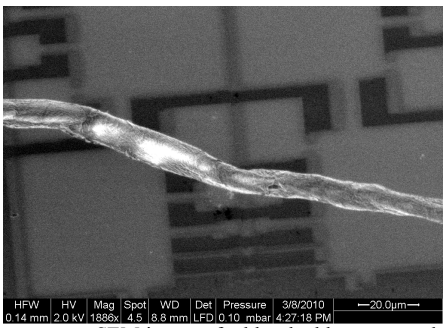


Fig 2. Low vacuum SEM image of a bleached but uncoated softwood kraft fiber in front of arbitrary contact pads. The biggest pads have a size of 50x50 µm and the diameter of the fibers is around 8 µm.

As described in the introduction and shown in Figure 1 two different biomaterials are used for our work. On the one hand, we use DNA to build flexible nanowires which have a semiconducting behavior but are hard to handle with a mechanical manipulation like gripping or AFM-based approaches. On the other hand, we use cellulose fibrils taken from wood fibers that are more robust than DNA but have no usable electric properties. In order to use them as nanowires a metallization is necessary. In addition, there are differences in the raw form of the used materials. While DNA is a long macromolecule which is coiled in liquid solutions, the fibrils are ordered in wooden fibers and have to be separated before they can be used. In the following sections we describe the necessary steps to use these two different biomaterials in order to realize the use as bonding wires for nanopackaging. Figure 2 gives an impression on the ratios between contact pads and wood fibers.

### *A. Nanopackaging using DNA nanowires*

As discussed earlier, one of the potential materials for bonding and packaging at the nanoscale is DNA. The physical and electrical properties of DNA enable the handling and usage of these biomolecules like classical bonding wires. For this, several crucial steps have to be performed to realize packaging. First, the DNA has to be immobilized in a way so that the strands can be used properly. This can be achieved by (i) stretching, (ii) immobilization and (iii) preparation of DNA wires with defined lengths. Today, different procedures for the (i) stretching of DNA are well-known and were already performed. Also, for the (ii) immobilization of DNA, on substrates like silicon and mica, methods are known for dry and liquid environments. For the (iii) preparation of nanowires, two methods are possible, depending on the automation grade necessary for later industrial use. The two methods are the enzymatic and the mechanical cutting of the DNA strands. Whereas the enzymatic method needs a special preparation step, which cannot be automated yet, the mechanical method can be performed fully automatically [22, 23]. The main drawback for the mechanical method is the additional time required to perform the manipulation using AFM-based approaches, whereas an enzymatic reaction is able to create a high number of defined parts in a very short time. The most crucial drawback of this method is the splitting of the DNA into various fragments (depending of the used enzyme) and demands an additional gel electrophoresis step. A gel electrophoresis is necessary to sort the nanowires. Once the wires are immobilized and ready to use, the handling steps can be performed using AFM-based ap-

proaches at dry conditions. For this, the tip of the AFM cantilever is functionalized to remove the DNA from the surface. The next step is the transport to the first contact or bonding pad, where an additional functionalization with special proteins is used for the binding of the DNA to these sites. With the AFM, the nanowire is stretched to the other, similarly functionalized, contact pad. There, the DNA will be placed onto the binding site. Necessary conditions for these methods are different binding forces of the used proteins for the functionalization of the contact pads and the cantilever tip. To support the removal of the DNA from the cantilever, additional negative voltages on the cantilever can be used. Depending on the nature of the electric circuit, the DNA can be metalized [10, 12] or used as a semiconductor [24].

### B. Nanopackaging using cellulose fibrils

The usage of fibrils of wood fibers for packaging purposes requires similar steps as DNA (see Figure 1). First, (i) the usable part needs to be separated from the fiber (separation step). During our first gripper-based experiments (see Section III.) it became obvious that specialized methods are necessary because of the high bonding forces. A solution is the use of metal tips to hold the fiber in place while using different pulling angles. Second, if the fibril is separated, it has to be transported and placed on both contact pads (handling and positioning steps). For this (ii) handling procedure, different properties of the fibril need to be considered. Fibrils are not as regular as any other organic material. In turn, their mechanical properties like bending stiffness are anisotropic. A possible automation system needs a calculation model to perform the planned actions. This requires characterization steps of fibrils during the first development stages for this type of nanopackaging. Third, it is likely that a fixation step is necessary during the (iii) oriented positioning, which could be solved by electrostatic means, until the fibril can be metalized. The fourth required step to turn the fibril into a nanowire is the (iv) metallization.

## III. Handling and Manipulation of DNA

The opportunity to manipulate DNA strands with an AFM tip was shown in many different experiments over the past years. Generally, most of them have been conducted under liquid conditions. In particular, the moving of DNA strands was implementable and shows very good results [25-31].

However, the dryer the environments gets, the stronger the interactions between DNA and the surface get, resulting in a high binding force. To enable a reliable design of nanoelectronic circuits using DNA or to use DNA for nanopackaging, proper methods to enable an automated handling at air or in vacuum have to be developed. For this, it is necessary to improve the immobilization of DNA. This section shows two alternative immobilization procedures which offer the potential of DNA manipulation under dry ambient working conditions.

### A. Sample preparation

#### 1) Materials

Double helix λ-DNA with a length of about 16 µm (48502 base pairs) is offered by Sigma Aldrich, Germany. It is extracted from Escherichia coli bacteria. The DNA-strands are stored in an ultra-pure water solution with a concentration of 1 µg DNA / 1 µl water. TE/$MgCl_2$-Buffer (10 mM TRIS, 1 mM EDTA with magnesium chloride) and AA/$MgCl_2$-buffer (20 mM ammonium acetate with 5 mM magnesium chloride) with a pH-value close to 7 were used as buffer systems for the following immobilization steps.

For surface modification, 9.9 mm diameter mica slices and commercial APTES (98%, Sigma Aldrich) were used. For pure immobilization 5x5 mm² silicon substrates were used.

For preparation, the mica substrate was fresh-cleaved each time before using. All silicon surfaces were cleaned with 2-propanol (isopropyl alcohol) and ethanol (99.6% pure ethanol) before using.

#### 2) Methods

Immobilization of DNA on silicon – The DNA solution was diluted in TE-$MgCl_2$-buffer (1 µl/1000 µl). 20 µl of this DNA/buffer mixture was dropped on a sheet of silicon and left there for 8 minutes. In this time, the DNA strands immobilized onto the silicon surface. With a piece of cellulose tissue the spare DNA/buffer solution was removed. This procedure absorbs the DNA/buffer mixture from the substrate and the DNA is aligned on the silicon surface by capillary forces. Because of the silicon hydrophobicity, further substrate dehydration is not necessary.

Immobilization of DNA on APTES-mica substrate – For APTES functionalization of mica substrates, 10µl APTES-solution (1% APTES in ultra-pure water) were left for 10 minutes on freshly cleaved mica. The APTES-solution cultivated a monolayer onto the mica surface during this contact time. Accordingly, the substrate was cleaned with 2 ml ultra-pure water and dried with nitrogen for 5 minutes.

For immobilization, the DNA was diluted in AA/$MgCl_2$–buffer (2 µl/1000 µl). 20 µl of this DNA/buffer mixture were dropped on the functionalized mica substrate and left for 10 minutes. After that, the substrate was cleaned with 2 ml ultra-pure water and dried for 10 minutes with nitrogen, again.

#### 3) AFM visualization and manipulation:

The implementations of the experiments were realized with a JPK NanoWizard II AFM. For visualization, the AFM was used in the intermittent-contact (tapping) mode. Manipulations and cuts were realized in contact-mode (automatic manipulation-mode). The AFM was equipped with a calibrated standard non-contact silicon cantilever from Nanoworld, without any coating. All experiments were con-

ducted at dry conditions and in air (T: 22-29 °C; max. 15% humidity).

### B. Results and discussion

#### 1) Silicon substrates

Figures 3 and 4 show DNA immobilized on a pure silicon substrate without any surface modification. Figure 3 shows the topographic AFM scan of the DNA strand (marked with an ellipse) with a height of about 1.5 nm. This strand is manipulated with the AFM cantilever tip in the following steps. Figure 4 shows the same DNA strand after several manipulations. The DNA was manipulated and successfully moved about 25 nm to 100 nm.

The transport of a single DNA strand via an AFM tip is shown in Figures 5-A and 5-B. This experiment demonstrates the complete removal of a single strand from the substrate surface. Figure 5-A shows two separate DNA strands. The long DNA strand on the top was manipulated with several manipulations up to 300 nm. Thereby, the whole DNA strand was removed from the initial location (see Figure 5-B).

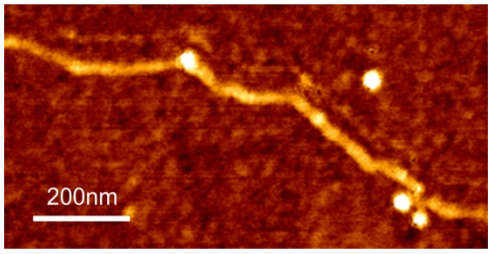


Fig 3. DNA immobilized on silicon without surface modifications.

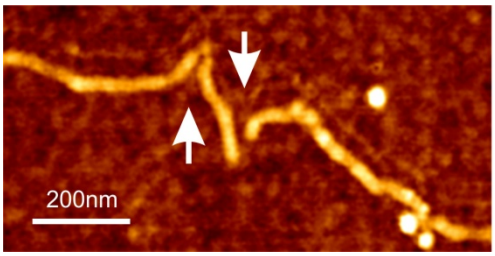


Fig 4. DNA immobilized on silicon after the manipulation process Two long distance movements, from bottom to top and from top to bottom, were implemented, resulting in DNA displacement by 100 nm. Additionally, two cuts across the strand were inflicted to handle the immobilized DNA strand in a controlled way.

#### 2) Modified mica substrates

This experiment verifies the potential of a strand manipulation in dry conditions. Figure 6-A shows a height image of an immobilized DNA strand on APTES-modified mica. Figure 6-B shows the same DNA strand after a manipulation with an AFM tip. The DNA strand was cut by the cantilever tip. An unintended damage of the APTES-layer is noticeable, but it is also possible to manipulate, cut and move the DNA strand without any destruction of the functionalized monolayer.

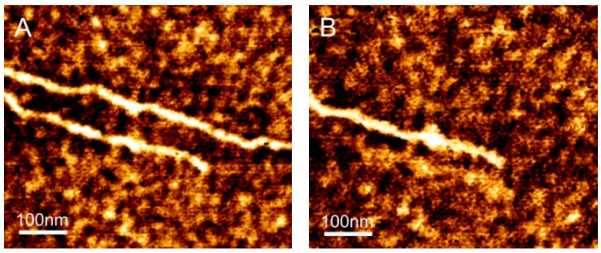


Fig 5. (A) Two different DNA strands immobilized on silicon without surface modifications. (B) The same substrate with the upper DNA strand removed from the surface by the AFM tip, without affecting the lower strand.

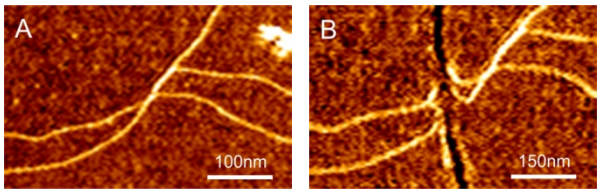


Fig 6. (A) DNA immobilized on an APTES modified mica surface. (B) The same immobilized DNA strand on APTES modified mica surface after manipulation by the AFM. The DNA strand was cut by the cantilever tip. The ends of the cut DNA strands were bent in the cutting direction up to 100 nm.

The current experiments demonstrate that defined DNA strands can be prepared by AFM-induced mechanical cutting. Furthermore, in all the experiments they can be moved and deposited on substrate surfaces to predefined locations. However, just at dry conditions it is important to use defined surface modifications such as APTES. All made experiments show preparation and handling methods successfully, working at humidity lower than 15% with a repeatability and reliability greater than 95% for a fine movement of up to 300nm and the complete removal of chosen DNA strands. Additional experiments using different defined modifications of the electronic charges on the surface have to be performed in the future. Furthermore, the deposition of suitable DNA strands to use such small strands as bonding wires for packaging at the nanoscale have to be tested. It is expected that all these methods will also work in the vacuum chamber of an SEM.

## IV. Handling and Manipulation of Cellulose Fibers

As mentioned earlier, the automation of processes dealing with irregular structures like biomaterials remains difficult. For cellulose fibrils no comparable data is available yet. Aiming at industrial processes, many steps, mentioned in Section II-B, need to be transformed from manual processing into automatable methods and techniques.

### A. Sample Preparation

Softwood kraft pulp was provided from INP-PAGORA, France. A small bundle of pulp fibers is disintegrated inside a Petri dish by adding deionized water. Next, the consistency of fibers is decreased to allow the fibers to suspend individually in deionized water. The individual pulp fibers are collected by using a pipette from the Petri dish, under an

optical microscope, and they are placed on an SEM specimen holder. The SEM specimen holder is kept at room temperature until the individual fibers dry out. Then, the individual fibers are gold coated in a thin-film sputtering process for 30 s. The thin-film deposition process is performed with minimum possible plasma energy to avoid burning the free ends of the fibers or fibril bundles.

### B. Experiments

#### 1) Setup

The entire experimental setup takes place inside the SEM. The setup is modularly designed and uses two positioning systems as main components: The first positioning system is used for coarse handling and consists of three linear axes offering a maximal stroke of 35 mm in x- and y-direction and 27 mm in z-direction and a rotary axis. Each axis is equipped with an internal optical positioning sensor for closed-loop positioning with an accuracy of 1 µm. The second positioning system is used for finer movements and consists of three linear axes equipped with internal capacitive positioning sensors, offering an accuracy of 1.5 nm in closed-loop mode. The combination of coarse and fine positioning systems unites long operation ranges with high position accuracy and is a well-proven setup design for micro- and nanomanipulation tasks [32].

Electrostatic grippers used in this experiment possess an actuated jaw and a quasi-static jaw equipped with internal force sensors (Femtotools). This facilitates monitoring the gripping process on the one hand, and performing force/displacement measurements on the other hand.

#### 2) Performed steps

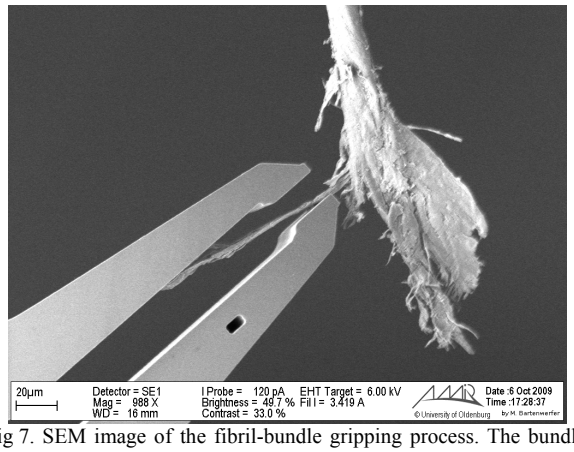


Fig 7. SEM image of the fibril-bundle gripping process. The bundle has a diameter of approx. 1.8 µm and a length of about 90 µm.

First experimental work on fibrils has been performed inside the SEM in high vacuum with pressures around $2 \cdot 10^{-6}$ mbar. Figure 7 shows the scene with the gripper bending a fibril bundle.

The length and thickness of the bundle in Figure 7 are 91.26 µm and 1.8 µm, respectively; and it is bended about 6 µm, successfully.

However, detaching the bundle from the fiber was not achieved.

### C. Results and discussion

The performed tasks indicate remarkable mechanical properties like strength, mechanical reversibility, and a very strong fibril-fiber joining. The strong fibril-fiber binding causes a combined bending of the bundle of fibril and the main fiber body of the aforementioned bundle of fibrils. Figure 8 shows the result of a force/displacement measurement on a fibril-bundle. Overall, the mechanical response of the fibril is linear and partly reversible.

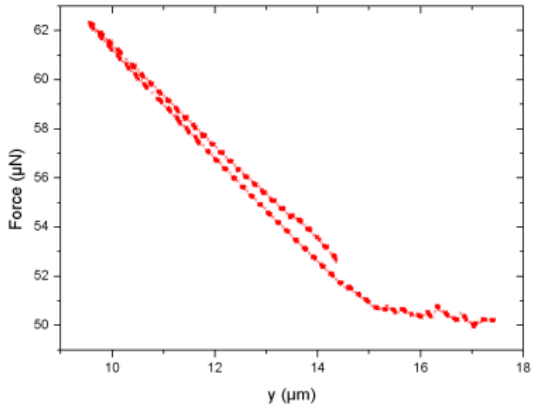


Fig 8. Force/displacement measurement on the fibril bundle shows a linear and reversible behavior.

## V. Conclusions and Future Works

The presented methods for the handling of biomaterials show the potential for different usage scenarios in industry and scientific research. In case of the handling of DNA, the experiments demonstrate that a DNA manipulation can be performed in dry ambient conditions. Both approaches (modified mica substrate and clean silicon surface) offer the opportunity to manipulate DNA properly. It is possible to cut, push and remove DNA strands from the substrate surface. By using these results and in combination with an AFM and nanorobots, this will lead to an automated solution of packaging problems at the nanoscale. However, upcoming work has to deal with the immobilization of the removed DNA as well as with the implementation of this method into a combined system of an AFM and nanorobots.

Concerning the work with cellulose fibers, the results motivate a more extensive investigation of the fiber-fibril structure in order to achieve a better understanding of the subject for possible future applications. At this stage, it is obvious that the use of cellulose fibers of wood and other sources depends on preceding characterization and manipulation tests to conclude the needed changes of the manipulating tools and systems. However, integrating an additional gripper or metal tip inside the SEM chamber to hold the wood fiber can be used to fixate the fiber properly and eliminate the effect of combined bending of the fiber and the fibril/fibril bundle. Furthermore, a more powerful gripper enhances the detachment of the fibrils from the main body of

the fiber. Different strategies have to be investigated in order to find proper parameters for the fibril detaching, handling and deposition. This is especially true regarding an automation of the processes. On this way, shear and tensile gripping approaches with diverse detaching angels and movement will be tested.

Wood fibers provide an economic and inexhaustible source of bio-nanowires and the results of this study confirm the potential of the nanohandling technologies in preparing and assembling fibril-based nanowires. In addition to the bio-nanowires, potential applications of automated nanohandling of wood fibrils include polymer nanocomposites [33] and characterizing biomass recalcitrance which is a key factor in biofuel production [34].

## VI. Acknowledgements

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