

Fabrication of Novel Nanomaterial Tungsten Disulfide (WS₂) Nanopores for Solid-State DNA Sequencing

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Tungsten disulfide (WS₂) is a novel nanomaterial that offers promise to create nanopores for easy and efficient detection of DNA translocation with high spatial resolution. In this work, nanopore membranes are fabricated, a method is designed for the transfer of WS₂, and WS₂ flakes are characterized for nanopore application and transferred to membranes. Semiconductive properties of WS₂ indicate promise for WS₂ nanopores, which are drilled in the WS₂ flakes. Silicon wafers were prepared using a standard method to form ~50 um x 50 um membranes for nanopore drilling. The created membranes were observed for uniformity and their size noted using optical microscopy. Scanning electron microscopy (SEM) was employed to visualize the membranes and focused ion beam (FIB) ~50 nm holes were sculpted. Flakes of WS₂ were acquired and images were captured using optical microscopy, height profile characterized by atomic force microscopy (AFM), and Raman spectroscopy used to observe monolayer or multilayer flakes. A stamp transfer method was designed to achieve optimal alignment of one flake to a membrane. Testing of the stamp transfer indicated a touch, release set-up that provided visual observation using optical microscopy. This provided the ability to visually align WS₂ flakes over membranes. Using minute motion, the flake was lowered to the microscope stage and contact was made with the membrane. The results of my contribution - wafer fabrication, SEM imaging, FIB drilled holes, designed stamp transfer, capturing optical microscopy images, atomic force microscopy (AFM) and Raman profiles for WS₂ flakes, and transfer of WS₂ to membrane indicate that the suspended WS₂ is ready for nanopore drilling. Once a nanopore is drilled in the suspended WS₂ flake, the Drndic group will conduct further experiments - Raman spectroscopy to check quality of 2D suspension, atomic force microscopy (AFM) to observe the suspended thickness and height profile, and methods to determine device noise and electronic frequency - to assist in determining the viability of WS₂ nanopores as gateways for DNA sequencing.

Background

There is a renewed interest in nanopores in the field of nanotechnology as a new, efficient way of sequencing DNA, and as a priority for improving personalized medicine 1-3. DNA translocation measurements are obtained as an applied field drives strands of DNA in salt solution through a membrane pore. As the bases pass through, a sensor reads each base. Nanopores provide a gateway for sequencing DNA, which is a powerful method to reveal genetic variations at the molecular level, including gene fusion and insertion/deletion, and is relevant to improving the understanding disease mechanism and genetic diagnosis 2.

Solid state nanopore sensors consist of thin, highly insulated membranes constructed from synthetic materials which proffer pore flexibility 1. Silicon nitride (SiNx), an insulator, is a common membrane material used for nanopore device fabrication 5. At the University of Pennsylvania, experiments in the Drndic Laboratory have demonstrated DNA and single molecule translocation through 2D materials like graphene, 4-10 and experiments in the Radenovic group have worked on molybdenum disulfide (MoS₂) nanopores 11. The major advantage of using 2D materials is that their signal is very high compared to the signal obtained from the nanopore membranes of thick materials like Si₃N₄ (thickness ~50-100 nm) 12, 13. Graphene nanopores are thin and flexible with good electronic conductivity and robust mechanical properties. However, graphene nanopores have relatively high noise; single nitrogenous bases are often

not detected or are detected improperly. Thus, applications requiring intermediate metallic behavior are better served by nanopores fabricated from semiconductor materials 1, 5. MoS₂ nanopores are an inorganic analogue of graphene with semiconductor properties that can be used for DNA biosensing 11.

Nanopore membranes fabricated from tungsten disulfide (WS₂), a novel 2D material, 14-16 can be used to detect DNA translocations. WS₂ is a semiconductor and member of the transition metal dichalcogenides group. The semiconductor properties of WS₂, similarly may allow for a viable alternative to graphene nanopore sequencing. MoS₂ and WS₂ have attracted attention from the scientific community due to their semiconductor band gaps, which permit intermediate metallic and insulating behavior due to strong covalent bonds and weak van der Waals stacking 16.

Flakes of WS₂ are being produced and studied, but nanopore experiments regarding electronic transport and nano-electric application have not been conducted with the material. Although MoS₂ and WS₂ are both members of the metal dichalcogenide group and have similar chemical properties, we expect to see differences in signal and noise characteristics.

Results and Discussion

Membranes were produced on SiO₂ wafers using the MA6 mask aligner lithography to pattern the wafer. Reactive Ion Etching was used to etch the patterned wafer. Using optical

Key Words: WS2 , fabrication, characterization, DNA sequencing, 2D-materials

Defined Terms:

Dichalcogenide - containing two atoms of chalcogen (group 16 elements) per molecule or unit cell; layered materials with strong in-plane bonding and weak out-of-plane bonding interactions enabling exfoliation into 2D layers

Semiconductor - material whose ability to conduct electricity is intermediate between that of a metal (conductor) and that of an insulator and is strongly temperature-dependent.

Translocation - substitution, displacement of a chromosomal segment to a new position, especially one on a nonhomologous chromosome

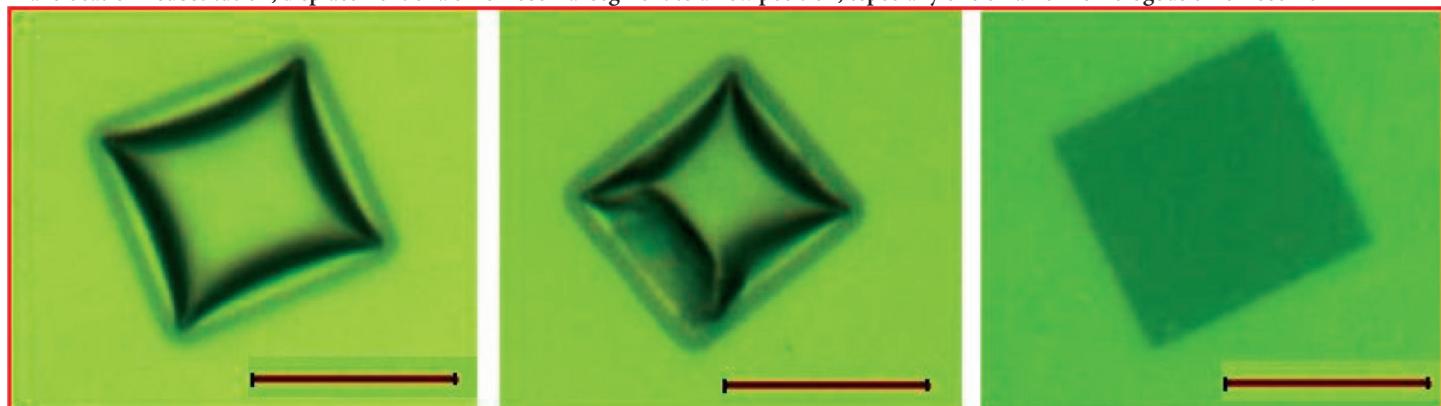


Figure 1: Optical microscope images of membranes illustrating side size lengths (um). On the left, the membrane is etched fully as shown by the symmetrical geometry of its interior. The middle membrane is half etched; it's right geometry matches that of the etched membrane on the left, but its left side is not symmetrical due to incomplete etching. The membrane at right unetched as seen from its lack of inner geometrical structure. All scale bars are: 34.13um.

microscopy, images of the membranes were captured, and sizes of the membranes were measured. Distribution of membrane size was graphed for perspective; average size of the membranes was ~50 um with standard deviation of ±4.2. The wafer was checked to see that the membranes were etched through. Membranes are etched through when an inner curvature is slightly risen on the square base. Images of the membranes were captured. Microscopy images illustrate that membranes were etched through and show membrane size (Figure 1).

Membranes were observed using scanning electron microscopy (SEM) and, using a focused ion beam (FIB), pores were drilled in the membranes. With the focused beam, pores were drilled - diameters ranging from ~20 - 100 nm - in the membrane windows. Due to the possibility of the beam stigmation if the FIB shook, some pores created were slanted rather than perfectly round. Adjusting the duration of the beam on the sample and HV could minimize slant. For the purpose of this experiment, it was not necessary to have perfectly round FIB holes. However, minimizing slant could contribute to decreased noise in translocation for future experiments. Minimal slant was observed in creating the 50 nm pores using 1pA for 1 second (Figure 2).

Flakes of WS2 used in this experiment were cultured by the Johnson lab at the University of Pennsylvania. Previously, in the Drndic lab, characterization and experiments were performed, creating nanopores with flakes of MOS2 . WS2 is a semiconductor structure similar to MOS2. Visualization of the WS2 molecular structures provides insight into its conducting behavior. WS2 exhibits van der Waals stacking between layers and laterally it exhibits strong covalent bonds laterally (Figure 3). The semiconductive nature of WS2 is contributed to the band gaps between layers promoting metallic behavior combined along with the insulating

behavior of the lateral bonding.

In preparation for the WS2 flake transfer, a method was designed to ensure precise transfer and positioning of a flake on a membrane. Traditionally, a chip with flakes of 2D material is placed on the wafer with membranes. The transfer is random, so flakes are not guaranteed to land on membranes. Using this method, if contact is made, placement can be skewed, in which case only part of the flake covers the membrane. To increase the chance that flake transfer is successful, a stamp method was designed (see methods). The method was tested with flakes of graphene. Graphene flakes on a chip were suspended on the PDMS gel (Figure 4). The PDMS with the graphene flakes easily separates from the chip in a copper solution. The gel with the graphene is secured to the tip of a micromanipulator. The micromanipulator, which was positioned adjacent to the optical microscope at an angle that positioned the tip of the manipulator with the gel under the microscope lens. Under 10x magnification, flakes were observed. The wafer with membranes was positioned beneath the suspended manipulator tip on the microscope stage; the two components were separate (Figure 6). Adjusting microscope focus, a membrane was identified. The fine motion of the micromanipulator allowed the gel to be lowered, and this. The lowering was observed on a screen hooked up to the microscope. Flake was touched to

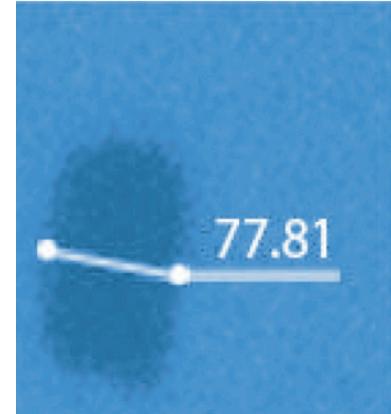


Figure 2: FIB hole 77nm in SiN 1pA for 1sec.

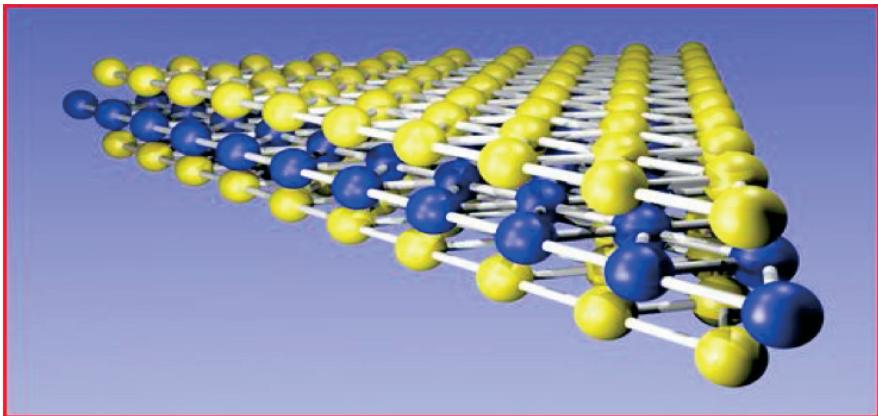


Figure 3: WS2 molecular structure exhibiting lateral bonding and stacking. Created by Paul Masih Das, Drndic lab using Blender

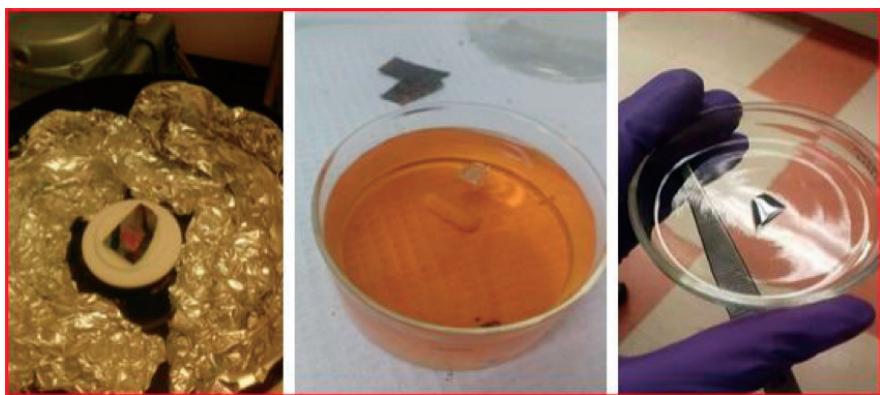


Figure 4: On the left, is a silicon chip with graphene flake placed on the spin coater; in the middle, the PDMA-chip separation is illustrated - the clear PDMS with graphene flakes lies on top of the copper solution, the wafer at the bottom of the solution; on the right, is the resultant PDMS gel with graphene flakes

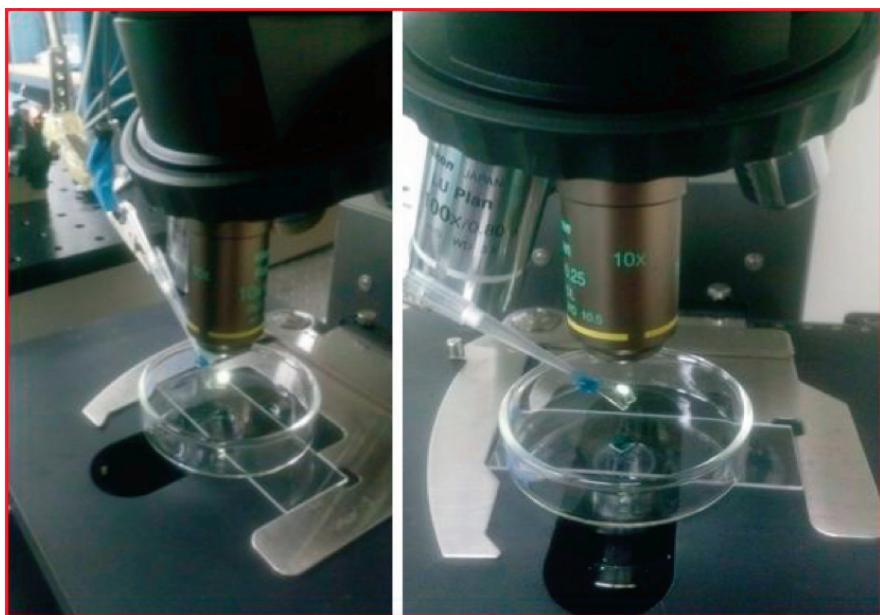


Figure 5: The micromanipulator was positioned adjacent to the optical microscope. Using minute motion, the apparatus is lowered to the microscope stage and the gel with flakes contacts with the membrane.

the membrane and transfer was complete. Testing of this method with graphene flakes demonstrates that this method will work with WS2 flakes.

Flakes of WS2 were imaged using optical microscopy. To capture flake sizes, a 10x magnification was used. Sizes of WS2 flakes averaged ~20 μm with a distribution ranging from a minimum ~7 μm flakes to a maximum of ~38 μm (Figure 6). Flake size depended on distance from tungsten seeds (Figure 7a). The Johnson lab cultured the WS2 pby placing tungsten seed on a wafer with sulfide. The largest flakes were imaged closest to the seeds. The largest flakes were mostly multilayer and were not ideal for this experiment due to their thickness. Single layer flakes of WS2 were found at a distance from the seed. Size distribution of the single layer flakes were recorded using the optical microscope measurement tools. Coloration of the flakes under the microscope indicated thickness. The thicker flakes were observed to be bright blue; single layer flakes were not bright. Clusters of thick and single layer flakes were observed on the sample (Figure 7b.). Ideal flake clusters were imaged on the sample edges and towards the center of the sample, areas which were further from the tungsten seeds (Figure 7c.). Once sizes were recorded using the 10x magnification, the 100x magnification was used. Images captured under 100x showed magnified features of the flakes (Figure 7d). Most magnified flakes showed perfect triangles, but however, the edges on some flakes were damaged. Damaged flake edges appeared like swiss cheese. For this experiment, the flakes with undamaged edges will be used.

Atomic force microscopy (AFM) showed a height profile of the WS2 flakes. Topography of the flake surface was characterized with AFM. A height profile was obtained as the AFM cantilever oscillated, scanning the surface of a WS2 flake. Generated noise from the scan and roughness of the WS2 flake corresponded to thickness of the flake. Height profiles were obtained for monolayer and multilayer WS2 flakes (Figure 8). Raman spectrometry was compared to known values for WS2 (Figure 9), which showed the monolayer and multilayer distribution of the WS2 flakes (Figure 10).

Transfer of the WS2 flakes to the membranes occurred using the designed stamp transfer method. The triangular

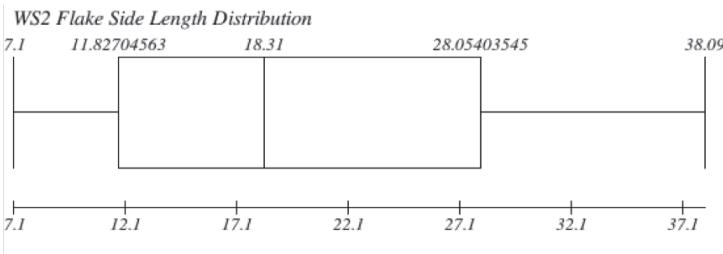
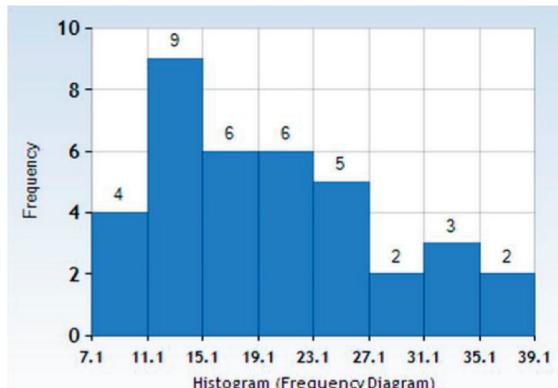


Figure 6: Top box and whisker plot and below histogram representations of size distributions of the side lengths of the WS2 flakes observed using optical microscopy



WS2 flake lays on the PDMS gel ready for transfer to the FIB hole in the membrane (Figure 11). Ultimately, transfer of flake to membrane proved unsuccessful. It is speculated that acetone was applied too soon to the PDMS with the flake once contact was made with the membrane. This did not allow the flakes to adhere to the membrane and leave the PDMS. In future experiments, a procedure will be designed where application of acetone is delayed so that the flake when the flake attaches to the membrane, it does so in a dry environment.

Materials and Methods

Wafer processing: SiO₂ wafers were prepared for this experiment. A Si wafer was cleaned using an O₂ plasma clean for 5 minutes on both sides. To wash the wafer, acetone was applied to each side followed by isopropanol. Wafer sides were dried with N₂. Resists were applied to the wafer through a spin coating process. The wafer was spin coated on one side with an S1818 positive resist at 4000 rpm for 45 seconds. Following spin coating, the wafer was baked at 115°C for three minutes, with the uncoated side on the hot plate. After baking, the wafer was spin coated on the other side with negative resist (NR7). The wafer was then baked at 115°C for three minutes, with the S1818 side on the hot plate.

Making windows using lithography and reactive ion etching (RIE): Using the MA6 mask aligner, one side of the wafer was exposed and patterned. The recipe was followed: 3.4 seconds at 365 nm at 5mW/cm². The wafer was baked at 115°C for three minutes. Development occurred by placing the NR7 side of the wafer in RD6 for 7 seconds. After 7 seconds, the wafer was rinsed with DI water and dried with N₂. RIE is used to etch the exposed nitride on the wafer. For a 50 nm wafer, etching occurred for 2 minutes; for a 100 nm wafer, etching occurred for 4 minutes. Following RIE, buffered oxide etching (BOE) was performed on the exposed SiO₂ layer. This layer was etched by subjecting the wafer to buffered oxide etching (BOE). The wafer was then submerged in a BOE bath. The wafer was tapped gently to remove bubbles and ensure the wafer surface was fully wet. The wafer was subject to the BOE for 70 minutes. After 70 minutes, an F-40 reflectometer was used to check if there was any SiO₂ remaining. If needed, additional etching time was performed to remove SiO₂. Once the F-40 reflectometer indicated the SiO₂ layer was removed, acetone was used

to strip photoresists. To etch the exposed silicon layer, a KOH etch was used. The KOH etch was prepared using a 40% potassium hydroxide (KOH) solution consisting of 1000 mL water and 666.67 g KOH. The solution was heated at 62°C and stirred at 120 rpm. An external probe monitored the temperature of the KOH solution. To etch 1 mm, KOH etching occurred for 22.5 - 23 hours. After 20 hours, the wafer was checked manually for visibility of light shining through membranes. When white light shined through the membrane, the etching was terminated. The wafer was cleaned using an acetone rinse followed by an isopropanol rinse. Membranes were observed using optical microscopy. S1818 photoresist was applied without spinning and let dry overnight to protect the unetched SiN layer. To remove the exposed SiN and SiO₂ layers, the wafer was placed in a HF BOE bath for 100 minutes. Following etching, acetone was used to strip the S1818.

Creating focused ion beam (FIB) holes: Using a focused ion beam, a focused beam of electrons was used to drill holes in membrane windows that ranged from 20 - 100 nm in diameter. The FIB machine was used following in accordance with a common procedure. The ion current was set to 2.2 ± 0.1 um, and t. The ion column HV was set to 5kV. Using a raster speed of 0.339 the membrane was scanned with SEM and positioned at a 52° angle. Angling the stage positioned the ion beam perpendicular to the membrane, allowing for the beam to enter straight on.

Transfer of WS2 onto FIB hole: In the interim of the Drndic lab acquiring WS2 flakes, a stamp-transfer process was designed to improve the transfer and achieve optimal alignment of one flake of WS2 onto a FIB hole. To test this method, a SiO₂ chip was used. On the chip, PMMA was spun at 1000 rpm for 30 seconds, and t. The chip was baked at 180°C for 3 minutes. PDMS mixture was made in a 10: 1 ratio of base to hardener. The PDMS acted as a gel that will be used to hold the flakes. PDMS was applied to coat the top of the chip and desiccated for 1 hour. Desiccation removed air bubbles. After 1 hour, the chip was removed and heated at 150°C for 10 minutes. A polyurethane quick cast was applied to secure the PDMS gel to a micromanipulator at a 30° angle. Once transfer had occurred, with the flake meeting the membrane, the gel was removed with acetone.

WS2 flake characterization: Flakes were characterized pre-transfer following optical microscopy, Raman spectrometry, and AFM protocols.

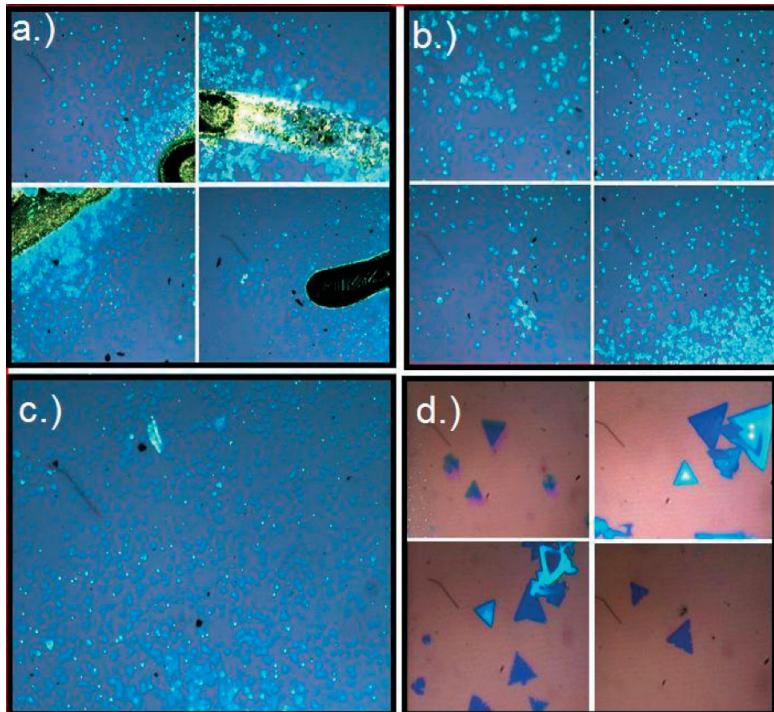


Figure 7: Optical Microscopy of WS₂ flakes. a.) Tungsten seeds (green/black) and surrounding WS₂ flakes. Further from the seed, the flakes are primarily monolayer. b.) Clusters of multilayer (illuminated a light blue) and single layer (less bright) WS₂ flakes. c.) Ideal, single layer WS₂ flakes. d.) 100x magnification of WS₂ flakes

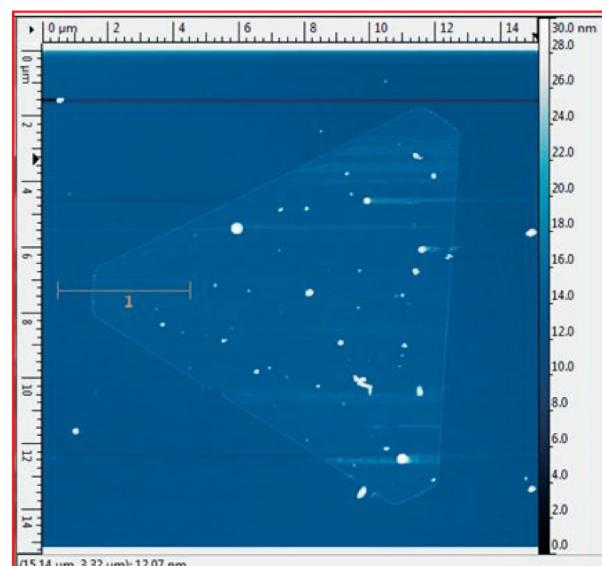


Figure 8: Atomic force microscopy (AFM) of WS₂ flakes. A flake is pictured with its width and height characterized. Generated frequency diagram corresponds to the flake's layer thickness.

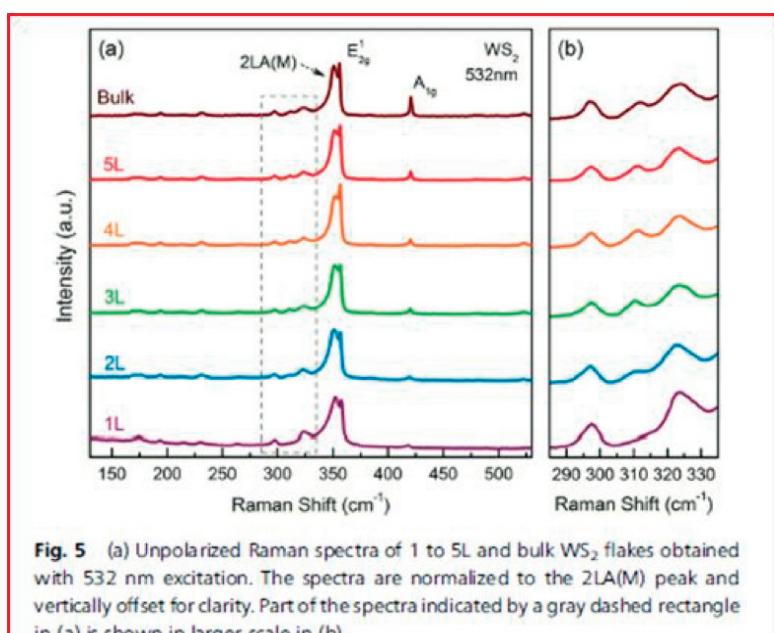


Fig. 5 (a) Unpolarized Raman spectra of 1 to 5L and bulk WS₂ flakes obtained with 532 nm excitation. The spectra are normalized to the 2LA(M) peak and vertically offset for clarity. Part of the spectra indicated by a gray dashed rectangle in (a) is shown in larger scale in (b).

Figure 9: Raman spectroscopy of WS₂ flakes. This is a diagram of the expected spectra for WS₂ with 532 nm layer. (Zhao et al. Nanoscale, 2013, 5, 9677 - 9683).

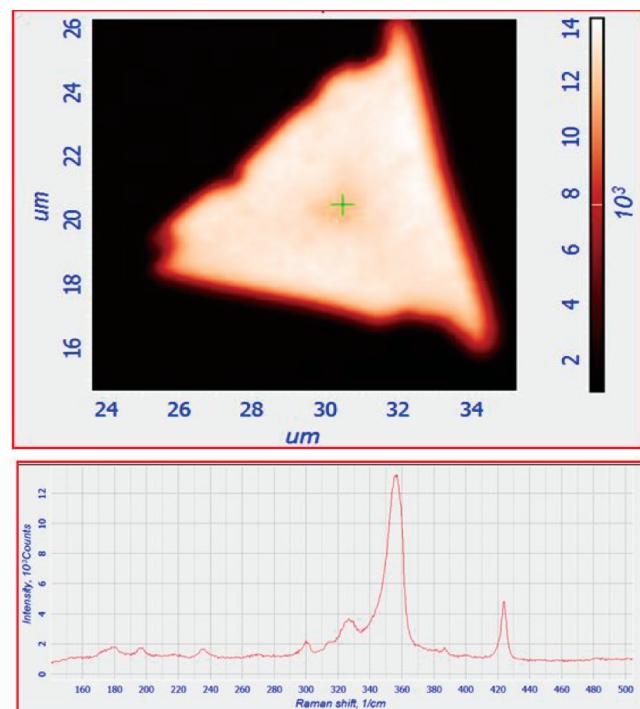


Figure 10: Raman spectroscopy. Based on the peak at roughly 310 cm⁻¹, our spectra is most likely 2 layers or more.

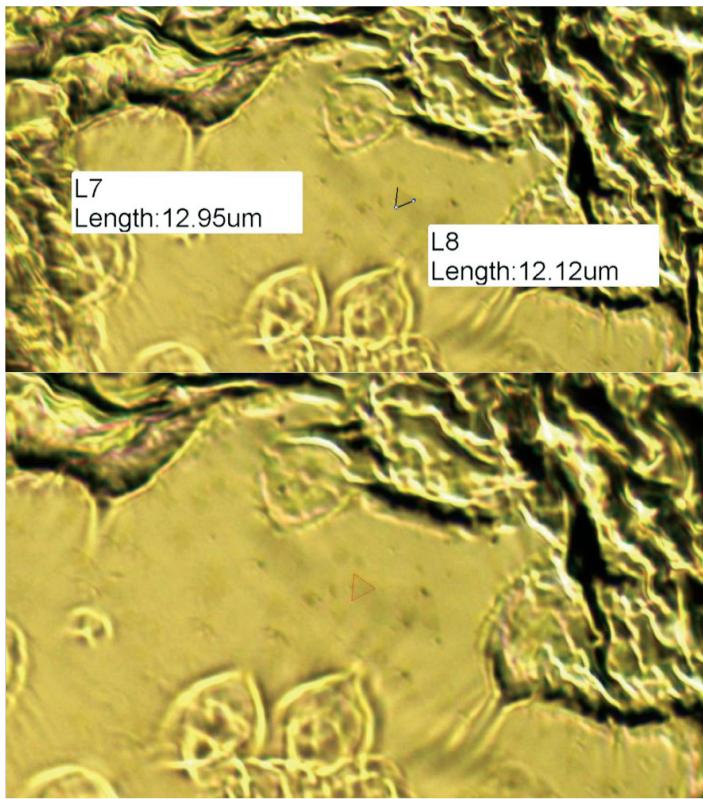


Figure 11: 10x optical microscopy images of WS₂ flakes suspended on PDMS. Flakes are ready for transfer.

Transfer of WS₂ onto FIB hole: A thin, PMMA gel was created to suspend the WS₂ flakes pre-transfer. The gel was placed on a microscope slide with WS₂ flakes open to air contact. The glass slide was attached to the micromanipulator at a 30° angle using the polyurethane quick cast. Once transfer had occurred, flake meeting the membrane, the gel was removed with acetone.

Conclusions

This research demonstrated fabrication of membranes, drilling of FIB holes, setup for the transfer of WS₂, AFM and optical microscopy of WS₂ flakes. In future work, once the transfer of WS₂ flakes has occurred, transmission electron microscopy (TEM) can be used to sculpt nanopores, Raman spectroscopy to check the quality of the 2D suspended membrane, atomic force microscopy (AFM) to observe thickness and height profile, and methods for determination of the device noise and electronic frequency employed. WS₂ offers potential for the creation of nanopores as an easy, efficient way to sequence DNA at a low cost. The development of WS₂ nanopores can be used to detect DNA translocation with high spatial resolution.

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