# Video Article Fabrication of Carbon Nanotube High-Frequency Nanoelectronic Biosensor for Sensing in High Ionic Strength Solutions

#### Girish S. Kulkarni, Zhaohui Zhong

Department of Electrical Engineering and Computer Science, University of Michigan - Ann Arbor

Correspondence to: Zhaohui Zhong at zzhong@umich.edu

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### Abstract

The unique electronic properties and high surface-to-volume ratios of single-walled carbon nanotubes (SWNT) and semiconductor nanowires  $(NW)^{1-4}$  make them good candidates for high sensitivity biosensors. When a charged molecule binds to such a sensor surface, it alters the carrier density<sup>5</sup> in the sensor, resulting in changes in its DC conductance. However, in an ionic solution a charged surface also attracts counterions from the solution, forming an electrical double layer (EDL). This EDL effectively screens off the charge, and in physiologically relevant conditions ~100 millimolar (mM), the characteristic charge screening length (Debye length) is less than a nanometer (nm). Thus, in high ionic strength solutions, charge based (DC) detection is fundamentally impeded<sup>6-8</sup>.

We overcome charge screening effects by detecting molecular dipoles rather than charges at high frequency, by operating carbon nanotube field effect transistors as high frequency mixers<sup>9-11</sup>. At high frequencies, the AC drive force can no longer overcome the solution drag and the ions in solution do not have sufficient time to form the EDL. Further, frequency mixing technique allows us to operate at frequencies high enough to overcome ionic screening, and yet detect the sensing signals at lower frequencies<sup>11-12</sup>. Also, the high transconductance of SWNT transistors provides an internal gain for the sensing signal, which obviates the need for external signal amplifier.

Here, we describe the protocol to (a) fabricate SWNT transistors, (b) functionalize biomolecules to the nanotube<sup>13</sup>, (c) design and stamp a polydimethylsiloxane (PDMS) micro-fluidic chamber<sup>14</sup> onto the device, and (d) carry out high frequency sensing in different ionic strength solutions<sup>11</sup>.

### **Video Link**

The video component of this article can be found at http://www.jove.com/video/50438/

#### Introduction

When a charged molecule binds to a SWNT or NW electronic sensor, it can either donate/accept electrons or act as a local electrostatic gate. In either case, the bound molecule can alter the charge density in the SWNT or NW channel, leading to a change in the measured DC conductance of the sensor. A large variety of molecules<sup>15-20</sup> have been successfully detected by studying DC characteristics of the nanosensors during such binding events. Even though charge-detection based sensing mechanism has many advantages including label-free detection<sup>21</sup>, femto-molar sensitivity<sup>22</sup>, and electronic read out capability<sup>15</sup>; it is effective only in low ionic strength solutions. In high ionic strength solutions, DC detection is impeded by ionic screening<sup>6-8</sup>. A charged surface attracts counter-ions from the solution which forms an electrical double layer (EDL) near the surface. The EDL effectively screens off these charges. As the ionic strength of the solution increases, the EDL becomes narrower and the screening increases. This screening effect is characterized by the Debye screening length  $\lambda_D$ ,

# $\lambda_D = \sqrt{\frac{\varepsilon k_B T}{q^2 c}}$

, where  $\varepsilon$  is the dielectric permittivity of the media,  $k_B$  is the Boltzmann's constant, T is the temperature, q is the electron charge, and c is the ionic strength of the electrolyte solution. For a typical 100 mM buffer solution,  $\lambda_D$  is around 1 nm and the surface potential will be completely screened at a distance of a few nm. As the result, most of nanoelectronic sensors based on SWNTs or NWs operate either in dry state<sup>20</sup> or in low ionic strength solutions<sup>5,15,17,21-22</sup> ( $c \sim 1$  nM - 10 mM); otherwise the sample needs to undergo desalting steps<sup>15,23</sup>. Point-of-care diagnostic devices need to operate in physiologically relevant ionic strengths at patient site with limited sample processing capability. Hence, mitigating ionic screening effect is critical for development and implementation of POC nanoelectronic biosensors.

We mitigate the ionic screening effect by operating SWNT based nanoelectronic sensor at megahertz frequency range. The protocol provided here details the fabrication of a SWNT transistor based nanoelectronic sensing platform and high frequency mixing measurement for biomolecular detection. The single-walled carbon nanotubes are grown by chemical vapor deposition on substrates patterned with Fe catalysts<sup>24</sup>. For our SWNT transistors, we incorporate a suspended top-gate<sup>25</sup> placed 500 nm above the nanotube, which helps enhance high frequency sensor response and also allows for a compact micro-fluidic chamber to seal the device. The SWNT transistors are operated as high frequency mixers<sup>9-11</sup> in order to overcome the background ionic screening effects. At high frequencies, the mobile ions in solution do not have sufficient time to form the EDL and the fluctuating biomolecular dipoles can still gate the SWNT to generate a mixing current, which is our sensing signal. The frequency mixing arises due to the nonlinear *I-V* characteristics of a nanotube FET. Our detection technique differs from the conventional techniques of charge based detection and impedance spectroscopy<sup>26-27</sup>. Firstly, we detect biomolecular dipoles at high frequency rather than the associated charges. Secondly, the high transconductance of SWNT transistor provides an internal gain for the sensing signal. This obviates the need for external amplification as in case of high frequency impedance measurements. Recently, other groups have also addressed biomolecular detection in high background concentrations<sup>23,28</sup>. However, these methods are more involved, requiring complex fabrication or careful chemical engineering of receptor molecules. Our high frequency SWNT sensor incorporates a simpler design and utilizes the inherent frequency mixing property of a nanotube transistor. We are able to mitigate the ionic screening effects, thus promising a new biosensing platform for real-time point-of-care detection, where biosensors functioning directly in ph

### Protocol

### 1. Catalyst Patterning for SWNT Growth

- 1. Begin with a silicon wafer with a low pressure chemical vapor deposition (CVD) grown 500 nm Si<sub>3</sub>N<sub>4</sub>/ 500 nm SiO<sub>2</sub> film on top.
- 2. Spin coat a layer of photoresist (PR) at 500 rpm for 5 sec and then 4,000 rpm for 40 sec.
- 3. Bake the wafer at 115 °C for 90 sec.
- 4. Use a photomask with rectangular pits for catalysts (Figure 1) and expose the wafer in UV (365 nm) irradiance of 300 mJ/cm<sup>2</sup> for 0.3 sec. After exposure bake the wafer at 115 °C for 90 sec.

**Tip:** Design pits of different sizes *e.g.* 5 micron x 5 micron, 10 micron x 5 micron etc. to account for the variability in SWNT chemical vapor deposition (CVD) growth process.

- 5. Develop the wafer in developer for 70 sec gently shaking the wafer through the process.
- 6. Rinse the wafer with DI water for 2 min and then blow dry with a nitrogen  $(N_2)$  gun.
- 7. Load the developed wafer into e-beam evaporator chamber and deposit 0.5 nm iron (Fe) at a chamber pressure of 10<sup>-6</sup> torr.
- 8. Dice the wafer into smaller dies 1.5 cm x 1.5 cm.
- 9. Remove the photoresist by dipping in warm acetone and isopropanol (IPA) for 10 min each. This leaves behind Fe catalyst in the rectangular pits for nanotube growth.

# 2. CVD Growth of Carbon Nanotubes

1. Place the catalyst coated dies in the quartz tube of the CVD growth furnace.

Tip: Determine sweet spot for nanotube growth. Growth is uniform over an area of 2inch x 2inch downstream for our furnace (Figure 2c).

- 2. Anneal the substrate in air at 880 °C for one hour to remove photoresist residue (Figure 2a). Allow to cool down.
- 3. Purge the chamber with Argon for 5 min at 3 SLM (standard liter per minute).
- 4. Ramp up the furnace to 800 °C at the center of the tube, while maintaining a flow of 1 SLM of argon (Figure 2b).
- 5. Flow 0.2 SLM of hydrogen for 5 min to reduce the catalyst particles *i.e.* convert iron oxide to iron.
- 6. Introduce 5.5 sccm (standard cubic centimeter per minute) of ethylene ( $C_2H_4$ ) for 35 min to grow SWNTs. Maintain a  $H_2$  flow of 0.2 SLM throughout the process. The length of SWNTs obtained from this recipe is > 20 micron ( $\mu$ m).
- 7. Allow the furnace to cool down to room temperature with a small argon flow.

# 3. SWNT FET Transistor Fabrication

1. Design a photomask (Figure 1) for defining source-drain electrodes for current-voltage (I-V) characterization of carbon nanotubes.

Tip: Extend electrode contact pads far apart on the die so that they remain accessible even after putting down a micro-fluidic stamp on the active nanotube region.

- 2. Follow steps 1.2 -1.6 to define area for metal deposition for contacts.
- <sup>3.</sup> Deposit Ti/Au 0.5 nm/50 nm for source-drain contacts in an e-beam evaporator chamber at 10<sup>-6</sup> torr.
- 4. Leave the die in acetone overnight for metal liftoff. After liftoff, dip the die in IPA for 10 min and then blow dry using N<sub>2</sub> gun.
- 5. Do a blanket deposition of 500 nm e-beam evaporated SiO<sub>2</sub> for gate dielectric at 10<sup>-6</sup> torr.
- 6. Design a photomask (Figure 1) for defining the gate electrode.
- 7. Follow steps 1.2-1.6 to define region for gate metal deposition.
- 8. Evaporate 50 nm/50 nm Cr/Au as the top gate electrode in e-beam evaporator. Follow step 3.4 for metal liftoff.

Tip: Use thick chrome layer to increase strength of suspended top gate. Gate dimensions are also critical for successful suspension.

- 9. Blanket deposit a thin layer (20 nm) of SiO<sub>2</sub> for top electrode passivation.
- 10. Design a photomask (Figure 1) to open a trench in SiO<sub>2</sub> to access the carbon nanotube channel. Design pad-etch area in the same mask to open up region for accessing source, drain and gate electrodes far away from the SWNT channel.
- 11. Follow steps 1.2-1.6 to pattern the PR to open up the trench for wet etch of SiO2.
- 12. Wet etch the evaporated  $SiO_2$  using 1:20 BHF solution for 3 min and 30 sec.  $Si_3N_4$  provides a etch stop layer to prevent further penetration of BHF.

Tip: Etch time calibration is recommended.

13. Thoroughly rinse the device in DI water, and then dip it in IPA for 5 min. Blow dry using a N<sub>2</sub> gun. The device structure remains intact due to the thick chrome layer.

### 4. Chemical Functionalization of Carbon Nanotube Sidewalls

- 1. Prepare a solution of 6 mM 1-pyrenebutanoic acid succinimidyl ester (PBSE) in dimethylformamide (DMF).
- 2. Incubate the SWNT FET die in this linker molecular solution for 1 hr at room temperature.
- 3. Thoroughly rinse the die in DMF to wash away the excess reagent. Blow-dry the device.
- 4. Prepare a 20mg/ml solution of biotinyl-3, 6-dioxaoctanediamine (biotin PEO amine -BPA) in DI water for biotinylation of SWNTs.
- 5. Incubate the die in this solution for 18 hr after which thoroughly rinse the die in DI water and blow dry. BPA attaches to the PBSE linker molecule.
- 6. Prepare a solution of 1mg/ml streptavidin in 7.2 pH PBS solution for streptavidin binding.
- 7. For static measurements, incubate the die in streptavidin solution for 20 min to completely functionalize the biotinylated SWNT. Thoroughly rinse and blow dry the die. For real time sensing, stamp the PDMS flow channel (step 6) first and then flow the streptavidin solution in high ionic strength background for streptavidin binding (**Figure 3**).

**Note:** We rinse the die by dispensing DI water (~50 ml) over the die using a squeeze bottle. Then we move the die to another Petri dish containing DI water and move the die around for 1 min. We repeat the two steps a total of 8-10 times.

### 5. Preparation of Polydimethylsiloxane (PDMS) Mold for Fluid Chamber

- 1. In a weighing cup, pour 9 parts by weight of PDMS monomer and add 1 part by weight of curing agent and thoroughly mix the two.
- 2. Degas the mixture in a desiccator for 25 min. The bubbles will rise through the mixture and leave.

Tip: If the mixture starts foaming, vent the chamber and let it settle down for a few seconds before degassing again.

- 3. Place a new silicon wafer in a Petri dish. Pour the degassed PDMS mixture on top of it to have a PDMS layer of 5 mm above the wafer.
- 4. Place the Petri dish in an oven at 70 °C for 1 hr.
- 5. Remove the Petri dish and let it cool down. Use a scalpel to cut out a rectangular piece of PDMS and pull it out using a tweezer.

Tip: The PDMS side directly in contact with the silicon wafer is clean and extremely flat. This side will be in contact with the SWNT FET die. Be careful not to contaminate it.

- 6. Place the rectangular die upside down and drill a hole in it using a biopsy punch (3 mm diameter) from the flat side to the other. This ensures no rough edges on the flat side of PDMS (Figure 4a).
- Place the PDMS chamber on top of the device carefully by aligning it on top of the active area of the fabricated SWNT FET dies (Figure 4a, b). Tap it gently to secure the stamp on die. The flat side bonds to the die to provide a leak-tight chamber.

**Tip:** This can be done with the naked eye or using an optical microscope with enough working space. If the PDMS does not stick well (generally if the die and/or the PDMS stamp is not clean), do oxygen plasma (20 watts, 15 sec) on PDMS to assist bonding. Using plasma powers higher than this leads to stronger bonding, however, we have seen ripping of electrodes while removing the PDMS in such case.

8. Remove the PDMS stamp after electrical testing and before next chemical functionalization step by dipping the die in DI water and gently lifting the stamp.

# 6. Preparation of Microfluidic Flow Channel

- 1. Take a clean silicon wafer and place it on hot plate at 200 °C for 5 min to remove any moisture.
- Spin coat SU-8 2015 at 500 rpm (100 rpm/sec ramp rate) for 5 sec and then 1,250 rpm for 30 sec at 300 rpm/sec ramp rate. This gives a 30 µm thick SU-8 layer on silicon wafer.
- 3. Soft bake the wafer at 95 °C for 5 min.
- 4. Design a photomask (Figure 4c) to define the 300 µm wide flow channel pattern on top of the SWNT area.

Tip: To avoid collapse of structure a channel width: height ratio of 10:1 is sufficient (300 µm: 30 µm in this case).

- 5. Using 365-nm UV photolithography define the flow channel with a UV exposure time of 0.9 sec.
- 6. Post bake the die at 95 °C for 5 min.
- 7. Develop the pattern in SU-8 developer for 5 min accompanied by gentle shaking.
- 8. Rinse the wafer in IPA and blow dry with  $N_2$  gun.
- 9. Follow steps 5.1-5.2 to prepare a PDMS mixture.
- 10. Place the wafer with SU-8 mold in a desiccator along with a 2-3 drop of silanizing agent trichloro(3, 3, 3-trifluoropropyl)silane in a Petri dish. Turn on the vacuum pump let the wafer sit in vacuum for 1 hr.
- 11. Pour the degassed PDMS mixture onto the wafer and heat it in an oven at 70 °C for 1 hr.
- 12. Cut the PDMS mold (negative of SU-8) using a scalpel.
- 13. Place the PDMS stamp upside down and using a biopsy punch (0.75 mm diameter) to drill a hole at each end of the flow channel. Make sure to drill the hole from the flat side (the side in contact with the silicon wafer) to the other to avoid rough edges (**Figure 4e**).
- 14. Place the PDMS flow chamber on top of the device carefully by aligning it on top of the active area of the fabricated SWNT FET dies under a microscope. Tap it gently to secure the stamp on die. The flat side bonds to the die to provide a leak-tight chamber. (Figure 4c and 4d)
- 15. Push a polyethylene tube into the holes and connect the other end to a fluid source and drain syringe (Figure 4e).
- 16. Attach the syringe to a syringe pump system to maintain a controlled flow of the fluid through the channel (Figure 6).

# 7. DC Electrical Measurement Setup

- 1. Connect the source and gate contacts of SWNT FET to voltage ports of a DAQ card.
- 2. Connect the drain contact to input port of DAQ card through a current pre-amplifier.
- 3. Apply 30 millivolt (mV) to source contact, sweep the gate voltage and record the current from the drain (Figure 5a).

Tip: For measurements in solution, keep gate voltage sweep parameter within |0.7 volts| to avoid leakage and reaction between the gate metal electrode and solution.

# 8. AC Electrical Measurement Setup

- 1. Connect the Ref-out signal from lock-in amplifier to the external modulation signal port on frequency generator to setup AM modulated frequency output.
- 2. Connect source contact of SWNT FET to the AM-modulated RF output port of frequency generator and dc voltage from DAQ card using a bias tee (Figure 5b and 5c).
- 3. Connect gate contact to voltage port of DAQ card.
- 4. Connect the drain contact to a lock-in amplifier to read the ac current through the nanotube. Read the amplitude and phase of current through the DAQ input ports.
- 5. Hold source dc voltage at 0 volt and AM signal frequency at 200 kilohertz (kHz).
- 6. Sweep the gate voltage and measure the current from the drain.
- 7. Increase frequency and repeat step 8.6 for I<sub>mix</sub>-V<sub>g</sub> sweeps at different frequencies.

# 9. Electrical Measurements in Solution (No Flow)

- 1. Prepare 1 mM NaCl, 10 mM NaCl and 100 mM NaCl salt solutions starting from 5M NaCl stock solution.
- 2. Take the SWNT device from step 3.13. Carry out steps 4.1-4.5 to obtain a biotinylated device.
- 3. Follow step 5 to put a PDMS chamber on top of the device.
- 4. Fill the chamber with DI water using a pipette.
- 5. Follow step 8 for frequency mixing measurements for different frequencies.
- 6. Repeat 9.4 for the three different salt solutions 1 mM NaCl, 10 mM NaCl and 100 mM NaCl.

Tip: Use pipette to withdraw previous solution and then flush the chamber multiple times with the new solution. Always switch from the low to high concentration solutions.

- 7. Remove the PDMS stamp by gently lifting the stamp with a tweezer.
- 8. Thoroughly rinse the device with DI water.
- 9. Carry out streptavidin binding as explained in 4.6-4.7.
- 10. Repeat steps 9.3-9.6.

# 10. Electrical Measurement in Solution (Real Time Flow)

- 1. Prepare 100 mM NaCl salt solution starting from 5 M NaCl stock solution.
- 2. Take the SWNT device from step 3.13. Carry out steps 4.1-4.5 to obtain a biotinylated device.
- 3. Place the micro-fluidic flow channel on the device following step 6.. Connect an empty syringe to one end of the micro-fluidic channel for withdrawal mode operation. At the other end attach a syringe with background solution of 100 mM NaCl.
- 4. Set up the electrical measurement as detailed in step 8. Fix the frequency (f = 10 MHz) and gate voltage bias (V = 0).
- 5. Start syringe pump in withdrawal mode (flow rate = 0.4 ml hour<sup>-1</sup>) and monitor the current signal with time. Switch the solution to 1mg/ml streptavidin in 100 mM NaCl and monitor signal change for streptavidin-biotin binding (Figure 6).

### **Representative Results**

A scanning electron microscope image of SWNT transistor with a suspended top gate is shown in **Figure 7a**. The gate dimensions are critical for suspension<sup>25</sup>. The current design dimensions are (length x width x thickness =  $25 \ \mu m \times 1 \ \mu m \times 100 \ nm$ ). The gate electrode consists of 50 nm Cr/50 nm Au; a thick chrome layer adds more strength to suspended structure. The suspended structure is confirmed by absence of leakage current between top gate and drain (**Figure 7b**).

We use the biotin-streptavidin ligand-receptor system to evaluate our SWNT sensor. To characterize the success of sidewall functionalization we monitor the FET DC transfer curves in air after each functionalization step. **Figure 7c** illustrate that the transfer curve shift to the right after biotinylation (red) and streptavidin binding (blue). This can be attributed to the electrostatic gating by the electronegative amine groups present on biotin PEO-amine and streptavidin.

For high frequency measurements, we follow the schematic shown in **Figure 5b**. The non linear I-V characteristics of SWNT transistor, mixes the high frequency inputs at the source and gate to yield a mixing current output,  $I_{mix}$  which is our sensing signal. **Figure 7d** shows  $I_{mix}$  measured as a function of gate voltage for a typical device in 100 mM NaCI. The mixing current for an AM modulated input at modulation frequency,  $\omega_m$ , is given by<sup>10-11</sup>

$$I_{mix}^{\omega_m} = -\frac{1}{4} \frac{\partial G}{\partial V_g} m v_{ac}^2$$

, where *m* is the modulation depth,  $v_{ac}$  is the AM input amplitude and  $\partial G/\partial V_g$  is the transconductance of the device (slope of the I<sub>dc</sub>-V<sub>g</sub> curve in **Figure 7d**). The mixing current results (*m* = 0.78 and  $v_{ac}$  = 20mV) agree well with the model as shown in the figure. For static fluidic measurements, we compare the peak of such mixing current sweeps for functionalized nanotubes. For flow measurements, we fix carrier frequency of AM modulated signal and fix gate voltage (V<sub>g</sub> = 0) and monitor I<sub>mix</sub> for biomolecular binding as a function of time, while maintaining a steady fluid flow. **Figure 7e-7f** shows the representative results for both static and flow measurements respectively.

For biomolecular detection, it is necessary that the CNT is exposed directly to the solution *i.e.*  $SiO_2$  is completely etched away during the BHF etch step. If this condition is not met, the chemical modification of CNT's is not possible as the linker molecule cannot stack along the nanotube sidewall. This is clearly illustrated in **Figure 7g** where we see no change before and after binding even in DI water for a  $SiO_2$  passivated device. This also proves that our measurement results indicate successful chemical modification as well as biomolecular detection in high background ionic concentrations. In all the measurements, we observe that the sensor response drops beyond 30 MHz which is due to resonance from setup.





**Figure 1. Nanotube transistor fabrication process flow. (a)** Fabrication process - (1) Photomask layer-1 (PL-1) for catalyst deposition, (2) metal liftoff, (3) CNT growth, (4) PL-2 for source-drain contact, (5) metal liftoff, (6) SiO<sub>2</sub> blanket deposition, (7) PL-3 for gate contact, (8) metal liftoff, (9) Thin SiO<sub>2</sub> blanket deposition, (10) PL-4 for BHF wet etch channel and (11) final device after photoresist removal. Color scheme is illustrated. **(b)** Schematic of device structure.



Figure 2. Carbon Nanotube growth. (a) Anneal step to remove photoresist residue, (b) growth step for CNT growth and (c) device placement in growth furnace.





Figure 3. Flowchart for chemical functionalization of CNT.

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**Figure 4. PDMS stamp for solution measurements. (a)-(b)** Static (no flow) measurements. **(a)** Punching and mounting a PMDS chamber on device, **(b)** Schematic diagram of flow chamber on a device. **(c)-(e)** Flow measurements. **(c)** Process flow for PDMS flow channel using SU-8 mold. (1) Photomask for defining flow channel, (2) cross linked SU-8 mold, (3) PDMS on SU-8 and (4) PDMS flow channel stamped onto device. **(d)** Schematic diagram of flow channel on a device and **(e)** Punching inlet/outlet holes in PDMS, stamping the flow channel on device and connecting polyethylene tubing to inlet/outlet ports.

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Figure 5. Electrical measurement setup. (a) DC measurement schematic, (b) AC mixing current measurement schematic and (c) image of experimental setup for AM modulated frequency mixing measurement. Click here to view larger figure.

### a.



b.

C.



Figure 6. Flow measurement setup. (a) Image of entire measurement setup; (b) Syringe pump and probe station; and (c) image of device with PDMS flow channel, inlet/outlet flow tubes and electrical probes.

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**Figure 7. Representative results for SWNT biosensor. (a)** SEM image of a typical suspended top-gate device, **(b)** gate-drain leakage to confirm suspended structure, **(c)**  $I_{dc}$ - $V_g$  curve for pristine nanotube FET (black), after biotinylation (red) and after streptavidin binding (blue) measured in air, **(d)** DC current,  $I_{dc}$  (black,  $V_{sd}$  = 10 mV) and mixing current,  $I_{mix}$  (red, modulation *f* = 200 kHz) as a function of  $V_g$  for the device in 100 mM NaCl solution. Theoretical  $I_{mix}$  obtained using the model in equation (1) is also shown ( $\blacktriangle$ ) for comparison. **(e)**  $I_{mix}$ - $V_g$  curves for biotinylated (black) and streptavidin-biotin bound (red) SWNT in 100 mM NaCl at *f* = 10 MHz, **(f)** real time flow measurement to detect streptavidin binding in 100 mM NaCl and **(g)** signal change after binding in a fully passivated control device in DI water at different frequencies. Click here to view larger figure.

### Discussion

The growth of carbon nanotubes depends not only on furnace conditions but also substrate cleanliness. The optimal gas flow rate, temperature and pressure for growth have to carefully calibrated and once fixed they are more or less stable. Even with these conditions being met, we found that growth depends on the patterned catalyst area, amount of catalyst and substrate cleanliness. Hence, we incorporated multiple catalyst pit sizes to account for the variability in growth. A one hour high temperature anneal step helped remove any contaminants like PR residue etc. from the substrate. **Figure 2** illustrates the conditions we adopted for SWNT growth.

The presence of contaminants from die processing steps may lead to spurious signal in chemical and biological sensing. Hence, it is necessary to clean the substrate thoroughly before and after chemical functionalization. The rinsing steps after each functionalization helps remove any excess reagent which may adhere to the device near the active area. We also observed that if the substrate was not clean, the PDMS stamp was not leak-tight and peeled off with fluid pressure during measurements. In such cases, very gentle  $O_2$  plasma on PDMS stamp helped adhesion. Too strong an  $O_2$  plasma may make the PDMS stamp stick well but hard to remove from the device; we have noticed ripping of electrodes while

removal which renders the die unusable. If the PDMS stamp and the die are clean, the adhesion between them is good enough to survive fluid flow measurements without oxygen plasma treatment also. Do not use O<sub>2</sub> plasma on the SWNT device as this will etch the carbon nanotubes.

In solution based electrical measurements, any leakage current overwhelms the detection signal. This leakage happens because the solution too can act as a conductor, the resistance of which goes down with increasing salt concentration. Therefore, it is necessary to incorporate electrode passivation step in the transistor design. The two blanket depositions in our fabrication protocol (500 nm and 20 nm SiO<sub>2</sub>) helped reduce leakage from source, drain and gate metal contacts. Also, before taking any electrical measurement, a gate-drain leakage sweep is recommended to ensure that no leakage happens in the intended sweep voltage range.

For fluid flow measurements, it is necessary to avoid air traps in the flow channel. The air gap leads to signal distortions because the sensor response in air is different than when in solution. In the forward pumping mode this issue was often encountered which impeded fluid flow. This was avoided by operating the syringe pump in withdrawal mode.

For the high frequency electrical measurements in high background ionic solutions the response dropped beyond 30-40 MHz due to the resonance loss from the measurement setup. We believe this can be improved by designing devices with lower parasitics. Optimized gate-SWNT distance and smaller BNC and SMA cables may help improve sensitivity.

### Disclosures

The authors declare that they have no competing financial interests.

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