Indication of Direction and Magnitude of Flow using DNA Nanotubes

by
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DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Abstract

In recent years, DNA nanotechnology has proven to be a rapidly expanding field brimming with scientific potential. Three-dimensional DNA nanostructures, known as DNA origami, are of particular interest for structural and diagnostic applications. This study established that a nanotube structure created by a DNA origami system could be used to indicate both the direction and magnitude of flow at the microscopic scale. Two different types of surface chemistries were considered for attaching DNA nanotubes: one for cells and one for a glass surface. Additionally, a flow system was designed that could test both. Cells demonstrated that they could withstand their respective attachment protocol inside the device and the glass attachment protocol was able to withstand shear stresses at and beyond a physiological scale. Ultimately, it was determined that longer nanotubes are better indicators of the magnitude of flowrate than shorter nanotubes. This study demonstrates that DNA nanostructures prove a more viable option than other potentially competing mechanisms for indicating the direction and magnitude of flow on the microscopic scale due to their modular nature and ability to self-assemble.
Acknowledgments

I would like to thank my advisor, Rebecca Schulman, for all of her guidance and feedback during my time at Johns Hopkins. I would also like to thank Sisi Jia for providing me the protocol for the specific attachment of nanotubes to HeLa cells, and for all of the help she provided with the various cell culture and microscopy techniques I employed in this study. I would like to thank Takanari Inoue’s lab for helping us develop the cell attachment protocol, and Konstantinos Konstantopolous’s lab for providing us with facilities, materials, and guidance while we were getting cell culture capabilities up and running in our own lab. I would like to thank Yi Li for developing the PEG to DNA conjugation that I used in my experiments, and I want to thank Mike Pacella for providing the initial code that I used in my studies, and for helping me to make my own adaptations to the code. Lastly, I would like to thank Jeffrey Gray for being my reader and for all of his valuable feedback on how to improve my paper.
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While DNA is known to contain information that translates to structure in biology through the central dogma of molecular biology, the physical properties of the macromolecule can allow the genetic code to directly influence the structure of the DNA itself in novel ways. Through Watson-Crick complementarity, DNA can self-assemble into different levels of structure, much like the way proteins can have up to four levels of structure. In the earliest demonstrations of this, a single long strand of DNA served as scaffolding that could be shaped into two-dimensional structures by the strategic placement of shorter single strands that served to “staple” the scaffold onto itself at specific locations.¹ Later studies showed that the scaffolding-staple strategy could be used to form three-dimensional structures, which is a critical component of this study.²

**Principles of DNA Origami Folding.**

The DNA nanostructures used in this study are organized into three increasingly complicated levels of structure. The first, most basic level describes the sequence of each DNA strand, which is simply some combination of the four elementary nucleotides. In the second level, complementary single strands of DNA can anneal to each other to form a structure that is determined by which strands anneal to which other strands. These structures make up the “building blocks” that will ultimately compose the third level of structure in this study: nanotubes that can grow up to and even past 10 microns in length.³

**DNA Nanotube Composition.**

Seeds and tiles, the two types of secondary structures formed in this study, are both formed under the same kinetic conditions, despite differing greatly in structure. The tiles in this study, called SEs tiles, consist of five types of single-stranded DNA sequences, labeled strands 1 through 5, that come together to form a “double ladder” structure. Strands 1 and 5 form the backbone of one ladder each, strand 3 (with a fluorescent label attached to it) connects both
ladders at the middle, and strands 2 and 4 connect both ladders at respective ends of the tile. Additionally, a few nucleotides on either end of both strands 2 and 4 remain unpaired to allow tiles to connect to each other as follows: overhangs on strand 2 can pair with overhangs on strand 4 in a diagonal pattern. The geometry of the tiles’ interconnection plays an important part in the third level of structure. The majority of the nanotube consists of bands of tiles that curve into rings, and these rings stack on each other to form each “tier” of the nanotube. Additionally, the number of exposed nucleotides can vary from experiment to experiment, and the length of the overhang, also called a “sticky end,” affects the optimal temperature at which the tertiary structure forms. Generally, the longer the sticky end, the higher the temperature at which stable nanotubes form.

Seeds consist of three major components. Most of their structure is made up of an M13 bacteriophage plasmid, they use FLIP staples to form the M13 into a tube-shaped structure, and they possess adapters that provide sticky ends on which tiles can connect. The way that the staples connect to the M13, a loop of the M13 hangs off, and this loop provides connection points for a fluorescent label. These seeds were developed because providing a nucleation point on which tubes can start growing results in an overall greater yield of nanotubes, but these seeds have an added benefit of being able to be serve as the anchor point for the nanotube. (See Figure 31 for greater detail.)

**Pre-existing Research in Flow Sensors.**

Several examples, both existing in nature and developed through research, may already address aspects of the specific challenge of this study: the indication of both direction and magnitude of flow. For example, hair cells found inside the inner ears of mammals are sensitive to mechanical perturbations caused by the movement of liquid, the frequency of which
contributes to the ability to hear.\textsuperscript{5} Another study conducted by Christopher Brockman, a Master’s researcher from the Schroeder group at the University of Illinois, established a mechanism to indicate flow using simple single- and double-stranded DNA.\textsuperscript{6} However, the modular system of interconnecting DNA that comprises the nanotubes in this study is far simpler than the former, and far more robust than the latter.

**Materials and methods**

**Development of a Flow Profile for Fluids in a Microfluidic Device**

This study required an analytical solution for the Navier-Stokes equation. This model would be used to calculate the velocity of fluid as a function of height from the floor of the device. Factors considered for this model were the dimensions of the device, the dynamic viscosity of fluid, and the volumetric flowrate of fluid through the device. The Navier-Stokes equation was adapted for the microfluidic device as follows:

\[
\rho \frac{D u}{D t} = F - \frac{\partial p}{\partial x} + \mu \frac{\partial^2 u}{\partial y^2}
\]

where \( \rho \) represents the density of the fluid, \( \frac{D u}{D t} \) represents the derivative of the velocity of the fluid with respect to time, \( F \) represents the force of the fluid per unit volume, \( \frac{\partial p}{\partial x} \) represents the partial derivative of the pressure drop with respect to the change in distance in the \( x \) axis (along the channel length), \( \mu \) represents the dynamic viscosity of the fluid, and \( \frac{\partial^2 u}{\partial y^2} \) represents the second partial derivative of the velocity with respect to the change in distance in the \( y \) axis (along the channel height).\textsuperscript{7} Because the kinematic viscosity of the fluid, \( \nu \), is equal to the dynamic viscosity divided by the fluid density, the equation could be modified as follows:

\[
\rho \frac{D u}{D t} = F - \frac{\partial p}{\partial x} + \rho \nu \frac{\partial^2 u}{\partial y^2}
\]
\[ \frac{Du}{Dt} = \frac{F}{\rho} - \frac{1}{\rho} \frac{\partial p}{\partial x} + \nu \frac{\partial^2 u}{\partial y^2} \]  

(3)

Furthermore, because the system would be at steady state, \( \frac{Du}{Dt} \) simplified to 0, and because of the inherently low Reynolds number of flow in microfluidics, \( F \) simplified to approximately 0 as well. Therefore, the overall equation simplified to:

\[ \nu \frac{\partial^2 u}{\partial y^2} = \frac{1}{\rho} \frac{\partial p}{\partial x} \]  

(4)

Because this simplification of the Navier-Stokes equation matched that of Iaccarino’s 2004 presentation,\(^8\) its model for the velocity distribution in the channel could be adapted for the purposes of this study. Its model is as follows:

\[ u = \frac{1}{2\rho_v} \left( -\frac{dp}{dx} \right) y(h - y) \]  

(5)

where \( h \) represents the height of the channel, \( u \) represents velocity of fluid at a distance of \( y \) from the floor of the channel, and \( \mu \) can be substituted for \( \rho_v \). Because of low Reynolds number, the \( \left( -\frac{dp}{dx} \right) \) expression could be simplified in the following way as such:

\[ \Delta P = \frac{a\mu Q L}{W H^3} \]  

(6)

where \( L, W, \) and \( H \) represent the length, width, and height of the microfluidic channel, respectively, and \( a \) represents a dimensionless term determined by the length and width of the channel. The term \( a \) is described as such:\(^9\)

\[ a = 12 \left[ 1 - \frac{192H}{\pi^5 W} \tanh \left( \frac{\pi W}{2H} \right) \right]^{-1} \]  

(7)

To get the velocity profile of fluid flowing through each microfluidic device, the above three terms were combined to reach a simplified expression \( k \):

\[ k = \frac{1}{2\rho_v} \left( -\frac{dp}{dx} \right) \]  

(8)

\[ k = \frac{\Delta P}{2\mu} \]  

(9)
\[ u = ky(h - y) \] (10)

where \( k \) in seconds\(^{-1}\) is only dependent on volumetric flowrate and the dimensions of the channel.

(See Figure 32 for greater detail.)

**DNA Nanotube Growth Protocol**

DNA nanotubes were grown using a two-step “double batch” procedure. The first batch contained the ingredients required to assemble the seeds, and the second contained the mixture of five tile strands.

**Conjugation of PEG to DNA.**

Two mixtures were first prepared in Dulbecco’s phosphate-buffered saline (DPBS), each containing its own unique single-strand sequence of amine-modified DNA at 50 µM and polyethylene glycol (PEG-AVS) at 2 mM. One sequence was the third strand of the tile mix, and the other was a strand that would attach to the seeds after assembly. Microcentrifuge tubes containing each mixture were vortexed gently for two hours, and the mixture containing the third strand of the tile mix was covered completely in aluminum foil because it was labeled with a Cy3 fluorescent tag.

Two polyacrylamide gel electrophoresis (PAGE) assays were prepared at 7 percent acrylamide for each solution of conjugated PEG-DNA. After vortexing, sufficient 6x loading dye was added to each solution to dilute the dye concentration to 1x, and each mixture was then divided into 20 µL aliquots to be loaded into its respective assembled PAGE assay. Electrophoresis was run at 150 V, using Tris-acetate-EDTA (TAE) buffered with magnesium at 12.5 mM as both the cathode and anode buffer. After one hour, each gel was removed from the caster, and the gel containing the PEG-conjugated sequence that would attach to seeds was immersed in a solution of SYBR® Gold dye in magnesium TAE for 30 minutes.
After staining, each gel was placed on an ultraviolet fluorescence table to identify the parts of the gel containing the PEG-DNA bands. These bands were cut from the gel, chopped finely, and transferred into clean microcentrifuge tubes. MilliQ (stringently filtered, deionized) water was added to the tubes at a sufficient volume to completely immerse the gel, and the tubes were completely covered in aluminum foil and vortexed gently for at least two days to dissolve the polyacrylamide. Both types of PEG-conjugated DNA were then quantified for use in nanotubes for the HeLa cell attachment protocol.

**Seed Growth and Purification.**

First, the single-stranded DNA required to form seeds was added into a microcentrifuge tube of MilliQ. Sufficient concentrated magnesium TAE buffer was added that the final concentration of magnesium would be 12.5 mM. FLIP staples were added at a concentration of 250 nM, 6-basepair adapters were added at a concentration of 100 nM, the M13 backbone was added at a concentration of 5 nM, the biotin-DNA linker strand was added at a concentration of 20 nM, Atto488 labeling was added at a concentration of 1 µM, and the seed-linker attachment strand was added at a concentration of 10 nM. For the glass slide attachment protocol, an additional ingredient, the biotin-conjugated DNA strand, was added at a concentration of 420 nM.

For both protocols, the DNA mixture was incubated for approximately three hours and fifteen minutes using Tube37 parameters in a thermal cycler. 50 µL of seed mixture total was grown, and no more than 20 µL were in each microcentrifuge tube. Once the thermal cycle was completed, the newly formed seeds were added to 350 µL of MilliQ water and transferred to a 100 kDa filter tube. The tube was centrifuged at 2000 rcf for 4 minutes, after which the filtrate was removed and an equivalent amount was added back into the filter. This step was repeated.
twice, and the filter tube was inverted into a clean tube. This new tube was further centrifuged at 2000 rcf for half a minute to collect the purified DNA seeds. These seeds finally were verified to be at a concentration of approximately 0.4 nM under a microscope. For the HeLa cell attachment protocol, the PEG-modified DNA strands was added after purification at a concentration of 0.9 μM.

**Nanotube Nucleation and Growth.**

The 5-strand DNA mixture required to form DNA tiles was grown using the same Tube37 parameters in a thermal cycler, and tiles with 6-basepair sticky ends were utilized so that optimal nanotube growth would occur at 37°C. Two tile mixtures were first assembled, one using PEG-conjugated, Cy3-labeled strand 3 and one using strand 3 that was only labeled with Cy3. The former mixture was used for nanotubes that would attach to HeLa cells, and the latter was used for nanotubes that would attach to a glass surface. For each tile mixture, strands were added to MilliQ water at such volumes that strands 1, 3, and 5 were at 1 μM and strands 2 and 4 were at 2 μM. For the purposes of any recipes that used these mixtures, they were considered to be at a simplified concentration of 1 μM.

In clean microcentrifuge tubes, sufficient concentrated magnesium TAE buffer was again added so that the final concentration of magnesium would be 12.5 mM. For tubes that would be attached to HeLa cells, the tile mixture was added at a concentration of 450 nM and the mixture containing the PEG-modified strand 3 was used. For tubes that would be attached to a glass slide, the mixture was added at a concentration of 150 nM.

After three hours and fifteen minutes of incubation, the strands had self-assembled into DNA tiles. As soon as the thermal cycler reached 37°C, seeds were added to the mixture at a concentration of 6 pM. The mixture of seeds and tiles was then left at 37°C to grow for at least a
day and up to a week. Nanotubes that would attach to HeLa cells were given at least three days to grow, as the PEG dramatically slowed down growth rate.

**HeLa Cell Culturing Protocol**

HeLa cells were first recovered from frozen storage. As soon as they were removed from liquid nitrogen, the cap on the vial was loosened slightly to let the pressure equalize. The vial was then gently swirled in water at 37° C for about a minute until a small amount of ice was left in the vial. The cells were pipetted into a cell culture treated T75 flask with 15 mL of warmed cell culture medium, and the flask was placed in an incubator at 37° C for no less 15 hours so the cells could settle and attach. The medium was finally replaced with HeLa cell medium, consisting of 1% penicillin and 10% fetal bovine serum in Dulbecco’s Modified Eagle Medium (DMEM), to remove any remaining DMSO in which the cells were frozen.

**Passaging HeLa Cells.**

HeLa cells were passaged when the confluency in the flask surpassed about 60%, or about every two days. A vacuum was used to remove the medium from the flask, which was quickly replaced with 3 mL of DPBS at 37° C to prevent drying out of the cells. The DPBS was then removed with a vacuum and replaced with 3 mL of 0.05% trypsin in EDTA at 37° C. The flask was put back in the incubator at 37° C for no more than 3 minutes to let the trypsin detach the cells from the bottom of the flask. The cells were then examined under a microscope to check if most cells were detached or could be easily detached by moving the flask around. If not, they were placed back in the incubator for another 30 seconds. If most cells were detached, the trypsin was neutralized by adding at least 7 mL of HeLa cell medium into the flask. A pipet was used to repeatedly flow medium over the bottom of the flask to ensure that as many cells were collected as possible, and the same pipette was used to transfer the cells to a separate centrifuge tube.
The cells were centrifuged for 5 minutes at 300 rcf and the supernatant was removed with a vacuum. Between 3 and 5 mL of HeLa cell medium at 37˚C was quickly added to centrifuge tube, depending on the size of the pellet remaining after centrifugation. The cells were resuspended and a small aliquot was set aside for counting. Once the cell density was determined using a hemocytometer, another aliquot was added to a new flask containing sufficient HeLa cell medium at 37˚C to dilute the aliquot to a concentration of 200,000 cells per mL.

**HeLa Cell Attachment Protocols**

At the last stage of passaging, an appropriate cell culture treated well plate or microfluidic device was inoculated with HeLa cells diluted to 160,000 cells per mL. The cells were then incubated at 37˚C overnight to allow attachment to the bottom of the well or channel. They were then stained with Vybrant™ DiD solution as per the protocol supplied by its manufacturer, Molecular Probes, after which a multi-step attachment protocol was performed, with the appropriate buffers for reagents aliquoted out beforehand. The DiD stain was imaged using a red fluorescent filter, as its absorption and emission spectra resemble those of the Atto647 fluorescent tag.

**Attachment Protocol for Well Plate.**

An unpublished attachment protocol, courtesy of Sisi Jia of Rebecca Schulman’s group, was provided for this following methodology. A cell culture treated 250 μL well plate was used to attach seeded DNA nanotubes to HeLa cells. After the cells were treated with DiD, they were put in 4˚C for 10 minutes to allow them to grow accustomed to the cold. The medium was then replaced with 1% m/V bovine serum albumen (BSA) in DMEM and the cells were put back in 4˚C. After 5 minutes, the cells were taken out and immediately placed over ice. From this point on in the protocol, every time the cells were removed from 4˚C, they were immediately put over ice and kept there to minimize the risk of endocytosis.
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The BSA in DMEM was replaced with the primary antibody, diluted to 2 μg/mL with 1% BSA in DMEM, which attached to the cells via the epidermal growth receptor (EGFR) overexpressed on the outside of their membranes. The cells were put back in 4°C and left for 30 minutes, after which they were washed with just DMEM. This consisted of a single fast wash and three slow washes, in which DMEM was kept in each well for 5 minutes while the well plate was gently rocked back and forth. After these four washes, the DMEM was replaced with a secondary antibody diluted 500 times with 1% BSA in DMEM. This antibody was conjugated to biotin and its antigen binding site was modified to bind to the primary antibody before addition to the system. The cells were then put back in 4°C, left for 30 minutes, removed from 4°C, and washed with DMEM over ice as per the previous step. This was then repeated with neutravidin, and then the biotin-conjugated DNA strand, each of which was diluted to 3 μg/mL and 1 μM, respectively, with 1% BSA in DMEM.

In the final step of the attachment protocol, after the four washes with DMEM, 200 μL of DNA nanotubes, plus 50 μL of 1% BSA in DMEM buffered with magnesium at 12.5 mM, were added to each well. The cells were then placed in 4°C for 2 hours and every 30 minutes, the wells were gently pipetted over ice. The buffered medium containing the unattached nanotubes was then removed and two washes were performed: one fast wash and another slow wash. (See Figure 33 for greater detail.)


The above protocol was performed up to the addition of the secondary antibody inside the channels of an Ibidi μ-Slide VI 01 ibiTreat microfluidic device.11 The secondary antibody was labeled with Atto488 fluorescent tag to verify attachment.
Experimental Setups.

Three experiments were conducted in the μ-Slide VI 0.1 device to test the efficacy of the nanotube attachment protocol for cells in a microfluidic environment.

**DiD Staining Cells in Device**

In the first experiment, cells were cultured, only stained with DiD, and subjected to flow to test the resilience of both the cells and the stain in the microfluidic environment. Cells were initially photographed in both visible light and using the red fluorescent filter. Luer adapters and silicon rubber tubing were then used to connect the microfluidic device to a syringe pump filled with HeLa cell medium. As Riva et al. suggest a flowrate of blood in the eye at about 40 microliters per minute, a flowrate at an appropriate magnitude needed to be considered. DMEM was pumped through the channel at 100 µL per minute for several minutes and pictures were taken in the same field of view, once again in both brightfield and the red fluorescent filter. Several new fields of view were then photographed in both filters.

**Intermediate Attachment Protocol in Wells and in Device**

In the second experiment, cells were first cultured in 250 µL well plates and the intermediate protocol was performed on them. In separate wells, the same protocol was performed but the primary antibody was not added. These wells served as the control group to verify that both antibodies functioned properly. Cells were imaged using a confocal microscope with both Alexa635 and Alexa480 filters used to image them in red and blue fluorescent light, respectively. The respective minimum brightness, maximum brightness, and gamma levels for each image’s respective fluorescent channel were noted and made uniform for comparison to the next set of images. This imaging procedure was then repeated on cells cultured in the μ-Slide VI.
device, with the minimum brightness, maximum brightness, and gamma levels on the images’ fluorescent channels set to the same respective values as in the previous set of images.

**Full Attachment Protocol Wells and in Device**

In the third experiment, the full cell attachment protocol was first performed in 250 μL well plate. In separate wells, the full protocol was performed without the biotin-conjugated DNA strand. These wells served as the negative control group. Cells were imaged using a confocal microscope with Alexa635, Alexa568, and Alexa480 filters used to image them in red, green, and blue fluorescent light, respectively. The respective black, white, and gamma levels were once again noted and made uniform for each respective fluorescent channel. Next, the full protocol was performed in the μ-Slide VI 0.1 device sans the DiD stain and cells were only imaged with green and blue fluorescent light. The minimum brightness, maximum brightness, and gamma levels on the images’ fluorescent channels were set to the same respective values as in the previous set of images.

**Glass Slide Nanotube Attachment Protocols**

An Ibidi μ-Slide VI 0.5 Glass Bottom microfluidic device was used to attach DNA nanotubes to a glass surface.13

**Surface-treating the glass with biotin-PEG-silane.**

The device was initially submerged in 10% m/V sodium hydroxide in MilliQ water and sonicated for one hour. The device was then rinsed with MilliQ water, submerged in methanol, and removed. Each channel was filled with 160 μL of 1% m/V biotin-PEG-silane in a solution of 95% methanol, 4% acetic acid, and 1% MilliQ water. The openings of the reservoirs were then sealed with parafilm to mitigate evaporation, and the biotin-PEG-silane was left to bond with the glass surface overnight, but not so long that the solution evaporated.
The next day, the solution of biotin-PEG-silane was removed from the channels and each channel was washed three times with methanol, keeping the glass surface hydrated at all times. Each channel then washed three times with water, and nitrogen gas was used to dry the channels completely. The device was placed in an oven at 70°C to bake for one hour, after which it was removed and each channel was filled with sufficient 1% m/V BSA in TNT buffer to cover the glass surface. Each channel was left with solution for 90 minutes and washed three times with TNT buffer, and then filled with neutravidin diluted to 0.4 mg/mL with 1% BSA in TNT. This solution was left in each channel for two hours, and each channel was then given three washes of TNT buffer followed by three washes of TAE buffer. Each channel was finally filled with 160 μL of TAE buffer and kept sealed with parafilm for up to a week until ready to receive biotin-linked, seeded DNA nanotubes.⁴

**Flow Experiments for Microfluidic Devices.**

The following equation from Ibidi documentation was used to determine the shear stress that would be experienced in the μ-Slide VI 0.1 device:¹⁴

\[
\tau \left[ \frac{\text{dyn}}{\text{cm}^2} \right] = \eta \left[ \frac{\text{dyn} \cdot \text{s}}{\text{cm}^2} \right] \cdot 10.7 \cdot \Phi \left[ \frac{\mu\text{L}}{\text{min}} \right]
\]  (11)

With a dynamic viscosity of 0.0072 dyn·s/cm², as assumed for cell media in the Ibidi documentation, and a flowrate of 40 microliters per minute,¹² a shear stress value of approximately 3.082 dyn/cm² was reached. To achieve a similar shear stress value in the μ-Slide VI 0.5 device, the following equation from Ibidi documentation was consulted:¹³

\[
\tau \left[ \frac{\text{dyn}}{\text{cm}^2} \right] = \eta \left[ \frac{\text{dyn} \cdot \text{s}}{\text{cm}^2} \right] \cdot 104.7 \cdot \Phi \left[ \frac{\text{mL}}{\text{min}} \right]
\]  (12)

With the dynamic viscosity of buffer assumed to be 0.01 dyn·s/cm², and a shear stress at 3.082 dyn/cm² or within one order of magnitude desired, flowrates of 0.16, 2.56, and 10.24 milliliters per minute were chosen for the following flow experiments. The flow profile that was previously
established in this study was then used to verify that the respective velocities of the fluid as a function of flowrate, height from the floor of the device, and dimensions of the device would be within one order of magnitude for respective heights in each channel.

*Initial Imaging of DNA Nanotubes Under Flow*

The initial goal for use of the μ-Slide VI 0.5 was to verify that nanotubes could withstand shear stresses from the flow of fluid at and beyond physiological levels. At least 60 μL of biotin-linked, seeded DNA nanotubes were grown for at least two days, added to each channel, and allowed to sit in the dark for at least 30 minutes. An additional 100 μL of magnesium TAE buffer was added to each channel to allow the fluid level in the channel to be high enough that an interface with no bubbles could form when the channel was connected to a flow system. Luer adapters and silicon rubber tubing were then used again to connect the microfluidic device to a syringe pump filled with TAE buffer, with special care taken to ensure that no air bubbles were trapped in the tubing. A field of view was chosen and buffer first was pumped through the channel at 0.16 milliliters per minute for several minutes. Videos were taken at a framerate of roughly 0.2 seconds using green fluorescent light to capture the Cy3-labeled nanotube tiles and a photo was taken in the same field of view using blue fluorescent light to capture the Atto488-labeled nanotube seeds. This was repeated for several more fields of view and for two other flowrates: 2.56 and 10.24 milliliters per minute.

*Analysis of Data from Flow Experiments.*

Once nanotubes could successfully be imaged under flow, a Python script, courtesy of Mike Pacella of Rebecca Schulman’s group (code is still in development, and thus as yet unpublished) was used to determine the absolute values of the angles of the nanotubes in flow through frame-by-frame analysis of each nanotube in each video. Canny edge detection was
performed on each frame, which removed noise from the image and used gradient detection to locate where on the single-color image the nanotubes could be found. The established gradient was used to create a skeleton of each nanotube, and an outline of each nanotube was created out of the skeleton based on a specified diameter, which helped to distinguish different nanotubes in the same field of view from each other. These outlines were filled in as masks, and the endpoints of each mask were used to compute both distance between endpoints and absolute value of the angle of the tube.

With the above script working for this study’s glass slide flow experiments, new videos of nanotubes experiencing the same previous three flowrates were taken. These videos consisted of alternating green fluorescent photographs and blue fluorescent photographs, and frames in the same channel were kept at least 15 seconds apart to minimize any chance of correlation between two consecutive angles for the same tube.

The Python script was then modified so that each nanotube detected in each frame was indexed and two arrays were created containing the nanotubes’ lengths and angles, respectively. For each set of data corresponding to each flowrate, nanotube angles were separated out by which angles corresponded to lengths shorter than and longer than a cutoff value of 3.5 microns. The data for each group of nanotube angles was then organized into a normalized frequency distribution with a bin size of one degree and fitted to a half-normal probability distribution function.
Results and Discussion

An Analytical Solution for Flow Profile in Two Microfluidic Devices

Flow Profiles for HeLa Cell Experiments.

The length, width, and height of the Ibidi μ-Slide VI 0.1 ibiTreat device are 17 millimeters, 1 millimeter, and 0.1 millimeter, respectively. As per Equation (7), the dimensionless factor $a$ was calculated to be about 12.80. Assuming a dynamic viscosity of cell medium of 0.0072 dyne-seconds per square centimeter, Equation (6) was first used to calculate pressure drop for a flowrate of 40 microliters per minute as 104.5 kilograms-second-squared per meter. Equation (6) was then used to calculate pressure drop for a range of flowrates from 6.25 microliters per minute to 200 microliters per minute and Equation (11) was used to calculate the shear stresses.

<table>
<thead>
<tr>
<th>Flowrate $Q$ ($\mu$L/min)</th>
<th>Pressure Drop $\Delta P$ (kg s$^2$/m)</th>
<th>Shear Stress $\tau$ (dyn/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>16.32</td>
<td>0.4815</td>
</tr>
<tr>
<td>12.5</td>
<td>32.65</td>
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<td>25</td>
<td>65.30</td>
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</tr>
<tr>
<td>200</td>
<td>522.4</td>
<td>15.41</td>
</tr>
</tbody>
</table>

*Table 1.* A summary of pressure drops in kilograms-second-squared per meter and shear stresses in dynes per centimeter squared as they pertain to a range of volumetric flowrates in microliters per minute in the Ibidi μ-Slide VI 0.1 device. The bolded value denotes the flowrate used in further experiments.

Using the pressure drops that were calculated for flowrates of 40 and 100 microliters per minute, as well as the dynamic viscosity $\mu$, the factor $k$ from Equation (10) was calculated to be $7.255 \cdot 10^4$ seconds$^{-1}$ and $1.814 \cdot 10^5$ seconds$^{-1}$ for a flowrate of 40 microliters per minute$^{12}$ and 100
microliters per minute, respectively. Thus, along with a height $H$ of 0.1 centimeter, profiles for these flowrates as functions of height from the floor of the device could be calculated.

<table>
<thead>
<tr>
<th>Height from Device Floor $y$ (μm)</th>
<th>Velocity $u$ (μm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowrate $Q = 40$ μL/min</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.183</td>
</tr>
<tr>
<td>2</td>
<td>14.22</td>
</tr>
<tr>
<td>3</td>
<td>21.11</td>
</tr>
<tr>
<td>4</td>
<td>27.86</td>
</tr>
<tr>
<td>5</td>
<td>34.46</td>
</tr>
<tr>
<td>Flowrate $Q = 100$ μL/min</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17.96</td>
</tr>
<tr>
<td>2</td>
<td>35.55</td>
</tr>
<tr>
<td>3</td>
<td>52.78</td>
</tr>
<tr>
<td>4</td>
<td>69.65</td>
</tr>
<tr>
<td>5</td>
<td>86.16</td>
</tr>
</tbody>
</table>

Table 2. Changes in velocity in meters per second as height from the floor of the Ibidi μ-Slide VI 0.1 device increases. These ranges were selected because both the heights of cells and lengths of DNA nanotubes can be expected to lie on the micron scale.

Flow Profiles for Glass Slide Experiments.

The length, width, and height of the Ibidi μ-Slide VI 0.5 device are 17 millimeters, 3.8 millimeters, and 0.54 millimeter, respectively. As per Equation (7), the dimensionless factor $a$ was calculated to be 13.17. Assuming a dynamic viscosity of buffer of 0.01 dyne-second per square centimeter, Equation (6) was used to calculate pressure drops for a range of flowrates from 0.16 milliliter per minute to 10.24 milliliters per minute and Equation (12) was used to calculate the shear stresses.
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

<table>
<thead>
<tr>
<th>Flowrate $Q$ (mL/min)</th>
<th>Pressure Drop $\Delta P$ (kg·s$^2$/m)</th>
<th>Shear Stress $\tau$ (dyn/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>0.9981</td>
<td>0.1675</td>
</tr>
<tr>
<td>0.32</td>
<td>1.996</td>
<td>0.3350</td>
</tr>
<tr>
<td>0.64</td>
<td>3.993</td>
<td>0.6701</td>
</tr>
<tr>
<td>1.28</td>
<td>7.985</td>
<td>1.340</td>
</tr>
<tr>
<td><strong>2.56</strong></td>
<td><strong>15.97</strong></td>
<td><strong>2.680</strong></td>
</tr>
<tr>
<td>5.12</td>
<td>31.94</td>
<td>5.362</td>
</tr>
<tr>
<td><strong>10.24</strong></td>
<td><strong>63.88</strong></td>
<td><strong>10.72</strong></td>
</tr>
</tbody>
</table>

Table 3. A summary of pressure drops in kilograms-second-squared per meter and shear stresses in dynes per centimeter squared as they pertain to a range of volumetric flowrates in microliters per minute in the Ibidi μ-Slide VI$^{0.5}$ device. The bolded values denote the flowrates used in further experiments. Of note is the shear stress value for a flowrate of 2.56 milliliters per minute as it is very close to the value of 3.082 dynes per centimeter squared obtained from a flowrate of 100 microliters per minute in the μ-Slide VI$^{0.1}$ device.

Using the pressure drops $\Delta P$ from Table 3 and the dynamic viscosity $\mu$, the factor $k$ from Equation (10) was calculated to be $4.991 \cdot 10^2$ seconds$^{-1}$, $7.985 \cdot 10^3$ seconds$^{-1}$, and $3.194 \cdot 10^4$ seconds$^{-1}$ for a flowrate of 0.16 mL/min, 2.56 mL/min, and 10.24 mL/min, respectively. Thus, along with a height $H$ of 0.54 centimeter, profiles for these flowrates as functions of height from the floor of the device could be calculated.
Table 4. Change in velocity in meters per second as height from the floor of the Ibidi μ-Slide VI \(0.5\) device increases.

Of note is the velocity profile for a flowrate of 2.56 milliliters per minute, as it largely matches up with the profile for a flow of 40 microliters per minute in the μ-Slide VI \(0.1\) device in order of magnitude.

Testing the Nanotube Attachment Protocol on HeLa Cells

Sisi’s attachment protocol, while shown to work for HeLa cells in 250 μL well plates in preliminary, unpublished results, required testing in the Ibidi μ-Slide VI \(0.1\) device to determine whether or not the protocol would be as effective in the device as in well plates. Due to the highly involved and time-consuming nature of this protocol, it needed to be broken down into several smaller protocols for microfluidic device.

**Testing Viability of DiD Stain and Cells’ Resilience to Flow.**

HeLa cells were photographed both before and after flow in both visible and red fluorescent light under the hypothesis that two minutes of flow at 100 microliters per minute
would not dramatically affect either the adhesion of the cells or the visibility of the stain on the cells.

**Figures 1, 2.** Photographs of two HeLa cells after staining with DiD fluorescent dye in the Ibidi μ-Slide VI 0.1 device. The photograph on the left was taken at 10x magnification, with a gain of 40 and an exposure time of 0.2 second, using a brightfield filter. The photograph on the right is of the same cells, taken in the same respective conditions, but using a red fluorescent filter. This set of photographs served as a control to which images captured after flow were compared.

**Figures 3, 4.** Photographs of the same HeLa cells after flow. The photograph on the left was taken with the brightfield filter in the same conditions as the previous respective photo. The photograph on the right was taken with the red fluorescent filter in the same conditions as in the previous respective photo.
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Figure 3 is nearly indistinguishable from Figure 1, with the exception of some peeling at the far edges of the cell due to flow, and Figure 4 still clearly shows an image of the cells in the red fluorescent light. Some washing out of the image is apparent, but that can be attributed to an observed tendency of Vybrant™ DiD to increase fluorescence as the amount of time exposed to the laser increases.

**Figures 5, 6.** Photographs of a new field of view in the same post-flow conditions. Figure 5 was taken with the brightfield filter and Figure 6 was taken with the red fluorescent filter.

Figure 5 shows most of one new cell and a partial image of another new cell clearly still adhering to the floor of the device despite some increased definition at the edges of each cell that suggest peeling. Figure 6 clearly shows images of each new cell in the red fluorescent light. There does not appear to be any washing out of the image, and the fact that only the central region of the main cell shows most of the fluorescence likely means that region just took up more dye.

HeLa cells were imaged in both 250 μL well plates and in the Ibidi μ-Slide VI 0.1 device using both blue and red fluorescent light under the hypothesis that results for cells in the microfluidic device would appear sufficiently similar to results in the well plates after applying the same intermediate antibody-EGFR protocol in both.
Figures 7-10. Images of HeLa cells stained with DiD and labeled with Atto488-conjugated antibodies in a 250 μL well plate. Cells were imaged under a red fluorescent filter, represented by pink, and a blue fluorescent filter, represented by green. The plots below each image represent the histograms for each fluorescent channel, with the color of each histogram matching the color of its respective fluorescent label in the image. Figures 7 and 8 on the top row depict cells upon which the intermediate protocol was performed with both antibodies added, and Figures 9 and 10 on the bottom row depict cells upon which the protocol was performed without the primary antibody. Each channel of each image possesses a minimum brightness value of 480, a maximum brightness value of 10,000, and a gamma value of 1.
The histograms depicting the levels for each red fluorescent image appear very similar to each other in size and shape. The histograms depicting the levels for the blue fluorescent images in Figures 7 and 8 are similar in size and shape both to each other and to the red fluorescent level histograms. The lack of green signal in either the images or the histograms of Figures 9 and 10 demonstrates that forgoing the primary antibody is an appropriate negative control for the next experiment.

**Figure 11.** Images of HeLa cells stained with DiD and labeled with Atto488-conjugated antibodies in the Ibidi μ-Slide VI 0.1 device. Cells were imaged under the same conditions as in the previous experiment, with the histogram colors and levels corresponding as well.
The clear presence of both pink and green in the image, as well as the size and shape of both histograms in Figure 11 suggest that both antibodies were able to bind to HeLa cells in the microfluidic device.

**Testing Full HeLa Cell Attachment Protocol.**

HeLa cells were imaged in both 250 μL well plates and in the Ibidi μ-Slide VI 0.1 device. Blue, green, and red fluorescent light was used to image cells in the well plates and only blue and green fluorescent light was used to image cells in the microfluidic device. Images were compared between the two cell culture systems to test the hypothesis that cells in the microfluidic device would appear sufficiently similar to results in the well plates after applying the same full antibody-EGFR protocol in both.
**Figures 12-15.** Images of HeLa cells stained with DiD and attached with DNA nanotubes, consisting of Atto488-labeled seeds and Cy3-labeled tiles, in a 250 μL well plate. Cells were imaged under a red fluorescent filter, represented by blue, a green fluorescent filter, represented by green, and a blue fluorescent filter, represented by red. Figures 12 and 13 on the top row depict cells upon which the protocol was performed with everything added, and Figures 14 and 15 on the bottom row depict cells upon which the protocol was performed without the biotin-conjugated DNA strand. The two figures on the left only show the red and green fluorescent signals and the two on the right only show the red and blue signals. Each channel of each image possesses a minimum brightness value of 500, a maximum brightness value of 2600, and a gamma value of 1. The yellow ellipses in Figure 12 depict detail not seen in Figure 13.
Two separate combinations of color channels were analyzed for each field of view because the overlaps between absorption and emission spectra for both DiD and Cy3 cause DiD to exhibit crosstalk with Cy3 when imaged under green fluorescent light. In comparing Figures 12 and 13, the regions of Figure 12 within the yellow ellipses show detail not seen in Figure 13, which demonstrates the presence of Cy3 in the image because it does not show up in the channel that picked up DiD. Although there is overall a very weak fluorescent signal in the red channel, the regions of red within the yellow ellipses that appear to intersect with the green signal, which suggests that the unique green signal corresponds to nanotubes that are attached to seeds. The lack of unique green signal in Figure 14 compared to the blue signal seen in Figure 15 demonstrates that forgoing the biotin-conjugated DNA strand is an appropriate negative control for the next experiment.
Figure 16. An image of HeLa cells attached with DNA nanotubes, consisting of Atto488-labeled seeds and Cy3-labeled tiles, in the Ibidi μ-Slide VI 0.1 device. Cells were imaged under the same conditions as in the previous experiment (minus red fluorescent light because no DiD was added), with the histogram colors and levels corresponding as well. The yellow ellipse shows a point of interest that may contain DNA nanotubes.

The green signal, while weak, still showed distinctive spots in the field of view that may be DNA nanotubes. The red fluorescent signal, while mostly showing the background fluorescence of the cells, also managed to pick up bright spots around the distinctive green areas as shown in the yellow ellipse.
Testing the Glass Attachment Protocol on DNA Nanotubes

Attaching DNA Nanotubes to a Glass-Bottomed Microfluidic Device.

DNA nanotubes were attached to the glass surface of the Ibidi μ-Slide VI 0.5 device and were subjected to three volumetric flowrates under the hypothesis that both the method used to attach DNA nanotubes to the glass surface and the DNA nanotubes themselves can withstand shear stresses at and beyond a physiological scale.

Figures 17-19. Glass-attached DNA nanotubes, consisting of Atto488-labeled seeds in the blue channel and Cy3-labeled tiles in the green channel, in the Ibidi μ-Slide VI 0.5 device. The figures depict, from left to right, nanotubes at flowrates of 0.16 milliliters per minute, 2.56 milliliters per minute, and 10.24 milliliters per minute. In each image, buffer is flowing from the right to the left.

While Figure 18 does not appear very different from Figure 19, Figure 17 is distinctive from both the following figures because the orientations of the DNA nanotubes on the image vary greatly, i.e. they are not all oriented from the right to the left due to diffusion from flow.

Canny Edge Detection and Development of Frequency Distributions.

New videos of DNA nanotubes were taken at the same previous three flowrates, but the frame rate was changed to 15 seconds between images. Once the frequency distribution script
was developed, distributions were developed for videos of corresponding flowrates under the hypothesis that a higher volumetric flowrate would result in a narrower frequency distribution.

**Figures 20, 21.** Result of Canny Edge Detection on a sample frame of a video depicting DNA nanotubes experiencing 2.56 milliliters per minute of flow in the Ibidi μ-Slide VI device. Figure 20 shows the actual photograph taken by the fluorescent microscope using a green fluorescent filter and Figure 21 shows the mask created by the Python script for this frame.

Although not every frame produces such clean results, Figure 21 captures nearly every nanotube from Figure 20 as its own distinct outline, with no false outlines created from noise in the actual image. The exception in this frame is the pair of nanotubes just below the middle of the image, which the algorithm detects as a single nanotube.
Figures 22-24. Initial histograms depicting the frequency distributions for nanotubes at three different flowrates in the Ibidi μ-Slide VI 0.5 device.

Figure 22 shows a wider distribution than either Figures 23 or 24, with a fewer relative number of angles at or near 0 degrees. Figure 24 shows a narrower distribution than either Figures 22 or 23 with very few angles not at or near 0 degrees. However, Figure 23 is much closer to Figure 24 than to Figure 22 in terms of the width of the distribution and the quantity of angles at or near 0 degrees.

Angle distributions were split into angles belonging to nanotubes shorter than or equal to 3.5 microns and angles belonging to nanotubes longer than 3.5 microns. These split sets of data were then normalized and fitted to half-normal probability distribution functions under the hypothesis that across a wide range of flowrates at the physiological scale, the distributions
created by longer nanotubes appear significantly different than those created by shorter nanotubes for shear stresses created by the same flowrate.

**Figures 25-30.** Histograms depicting the normalized frequency distributions for nanotubes at three different flowrates in the Ibidi μ-Slide VI \(^{0.5}\) device. The black line signifies a fit to the half-normal probability distribution function for each set of data.
Division of each set of angles to those belonging to longer tubes and those belonging to shorter tubes further exacerbated the differences between nanotube angle distributions for different flowrates, at least for the data sets corresponding to longer tubes. For this data, the probability distribution begins at a higher value as the rate of flow increases, and the difference in the width of the distribution for shorter versus longer nanotubes increases greatly as the rate of flow increases.

<table>
<thead>
<tr>
<th>Flowrate $Q$ (mL/min)</th>
<th>Shear Stress $\tau$ (dyn/cm$^2$)</th>
<th>Location Parameter</th>
<th>Scale Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For Tubes Shorter than or Equal to 3.5 $\mu$m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.16752</td>
<td>-6.6231E-10</td>
<td>29.082</td>
</tr>
<tr>
<td>2.56</td>
<td>2.68032</td>
<td>-7.1054E-15</td>
<td>19.650</td>
</tr>
<tr>
<td>10.24</td>
<td>10.72128</td>
<td>-1.3323E-14</td>
<td>21.605</td>
</tr>
<tr>
<td><strong>For Tubes Longer than 3.5 $\mu$m</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.16752</td>
<td>-6.7865E-08</td>
<td>13.050</td>
</tr>
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<td>2.56</td>
<td>2.68032</td>
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<td>10.24</td>
<td>10.72128</td>
<td>-1.3354E-09</td>
<td>5.642</td>
</tr>
</tbody>
</table>

Table 5. Location and scale parameters for the fittings of normalized distributions to half-normal probability distribution functions.

For each flowrate, fittings of angle distributions that were produced by longer nanotubes resulted in lower respective location and scale parameters than fittings that were produced by shorter nanotubes. Furthermore, as flowrate increased for all three sets of angles belonging to longer nanotubes, respective location parameter increased whereas respective scale parameter decreased. For angle distributions produced by shorter nanotubes, no discernible trend could be observed.
Conclusions

Overall, a system for indicating both the direction and magnitude of flow in a microfluidic environment using DNA nanotubes was successfully developed. The modularity of this system proved useful, as any one of a variety of modifications could be made to the DNA components of the nanotubes. Strands could be modified with PEG, one of several fluorophores, or important linker molecules such as biotin. While the DNA in Brockman’s study could likely be modified in similar ways, the simplicity of that design would likely prove limiting. Moreover, the study only shows DNA extending from a coiled conformation under flow, with no indications in the study that the conformation would change significantly under varying magnitudes of flow. The design and modification of the constituent components of this system can also be done relatively practically and affordably when compared to the amount of effort that would be necessary to utilize hair cells in the same way.

A flow profile for the transport of fluid through a microfluidic channel was successfully developed. If the dimensions of the channel, hydrodynamic properties of the medium, and volumetric flowrate are known for a given steady-state flow, then both the shear stress experienced on the floor of the device and the change in velocity of the fluid as distance from the floor of the device increases can be calculated. The model verified that two types of microfluidic devices could be used to produce shear stresses and velocities on the same physiologically relevant order of magnitude, as shown in Tables 1 through 4, by simply varying flowrate to account for differences in medium and channel dimensions. Additionally, this profile can easily be adapted in vivo if the use of anchored DNA nanotubes is desired for diagnostic applications.

A protocol that would allow DNA nanotubes to anchor themselves to cells that sufficiently express EGFR was successfully adapted for microfluidic devices. In Figures 3
through 6, HeLa cells were shown to be able to withstand shear stresses at a physiologically relevant scale, as was Vybrant™ DiD red fluorescent stain, in the Ibidi μ-Slide VI 0.1 ibiTreat microfluidic device. This demonstrates that HeLa cells are an appropriate model for which this attachment protocol can be tested and that DiD is a suitable stain for identifying cells under a fluorescent microscope in a microfluidic environment. The similarities in size and shape of the histograms for Figures 7, 8, and 11 also demonstrate that each step of the protocol up to and including the addition of the secondary antibody could be successfully carried out in the μ-Slide VI 0.1 device.

Although Figure 12 shows that the full EGFR-antibody protocol can be successfully performed on HeLa cells in 250 μL well plates, the yield was very low, with only one or two nanotubes at a time attaching to cells and very few cells displaying attached nanotubes in the first place. This could possibly be attributed to endocytosis of the attaching molecules by the cells, which despite being slowed by the cold would still occur if the protocol took too long to complete. Figure 16 further demonstrates that the full protocol produced a very low yield of nanotubes attached to cells, as within the μ-Slide VI 0.1 device, only a single attached nanotube even comes close to being clearly visible in a field of view that contains many cells. Working in the microfluidic device is significantly more tedious and difficult, which can contribute to the protocol taking even longer and the cells having even more time to endocytose the attachment mechanisms.

Nanotubes could attach to the μ-Slide VI 0.5 Glass Bottom device much more easily than they could to the μ-Slide VI 0.1 device. Although Figures 12 through 16 show very few, if any, nanotubes attached, Figures 17 through 19 show a much greater relative yield of nanotubes attached to the surface in terms of number of nanotubes attached per field of view. Additionally,
nanotubes in Figure 17 appear different enough from those in Figures 18 and 19 that given a large enough difference in flowrate, even qualitative analysis can be sufficient in determining amount of shear stress experienced by nanotubes in flow, even if that alone is not enough to determine the exact amount of shear stress experienced. Figure 19 specifically shows that DNA nanotubes can withstand shear stresses both at and comfortably beyond physiological magnitudes.

Figure 21 shows that Canny Edge Detection was a very successful first step in developing a quantitative measurement of flow. Figures 22 through 24 then show that the distribution of angles for nanotubes that experience higher flowrates became narrower as predicted. When these data sets were then split into angles belonging to shorter nanotubes (Figures 25, 27, 29) and those belonging to longer nanotubes (Figures 26, 28, 30), and when probability distribution functions were fitted to each newly separated data set, the functions for distributions of angles associated with longer nanotubes appeared significantly different than those for distributions associated with shorter nanotubes when comparing respective data sets. That Figures 26, 28, and 30 demonstrate such a clear narrowing of the probability distribution function as flowrate increases compared to Figures 25, 27, and 29 suggests that nanotubes longer than 3.5 microns provide distributions of angles under flow that can better indicate magnitude of flowrate than those provided by shorter nanotubes at a range between 0.16 and 10.24 milliliters per minute. Table 5 supports this even further, as a clear trend in both parameters would be necessary to produce a fitted model that changed predictably as shear stress experienced by nanotubes changed, and the distributions produced by longer nanotubes satisfy this requirement much better than those produced by shorter nanotubes.
Appendix

Supplementary Images

**Figure 31.** Overall schematic of the structure of the DNA nanotube. The M13 strand is shown in grey, the FLIP staples are shown in orange, the adapter strands are shown in yellow, and the tile strands are shown in green.\textsuperscript{3,4}

**Figure 32.** Diagram showing the shape of the flow profile caused by flow at a physiological scale in a microfluidic device. The $x$-axis denotes distance along the length of the device, and the $y$-axis denotes distance along the height of the device.\textsuperscript{5}
**Figure 33.** Overall schematic of the full HeLa cell nanotube attachment protocol.

**SEs nanotube tile sequences:**

SEs_1: \(\text{TCAGTGGACAGCCGTTCTGGAGCGTTGGACGAAACT}\)

SEs_2: \(\text{CCAGACAGTTTCGTGGTCATCGTACCTC}\)

SEs_3-5'Cy3: \(\text{/CY3/CCAGAACGGCTGTGCTAAACAGTAACCGAAGCACCAACGCT}\)

SEs_4: \(\text{GTCTGGTAGAGCACCACTGAGAGGTA}\)

SEs_5: \(\text{CGATGACCTGCTTCCGGTTACTGTTTAGCCTGCTCTA}\)

/Cy3/ denotes Cy3 fluorophore covalently attached to the 5’ end of DNA.

**The architecture of PEG-conjugated SEs tiles:**

SEs_Paint_5Cy3_3maine:/Cy3/CCAGAACGGCTGTGCTAAACAGTAACCGAAGCACCAACGCT

CGCTTTTT/AmMO/

/AmMO/ denotes amino covalently attached to the 3’ end of DNA.
Seed staple sequences:

stp_80_1: TGTCACGGTTCAGCCCTCTACCCGCACCACCCATCAGATCCGACTACGC
stp_80_2: GATGCCGGTTTCAAGACGACCTTAAATAAAACCGAAGACTATTTAGATCTCCAGACAGAAGATCGCCGACTACGC
stp_80_3: CAAGCAGTTTTCTTGGTGGAAGTGTGTTTTGCTAATACGCCACAAGCCTGTCGCAAGAAGATCCGACTACGC
stp_80_4: ACACCAGTTCTCATTAAACACATACCCACCATTTAACCTCAAGCCCTGTAAGATCCGACTACGC
stp_80_5: GGAACAGTTGAAACGCAACTAAACTACACATCTCTGGAACTTCCGGTGAAGATCCGACTACGC
stp_80_6: TGTGTCGGTTTCTGAAATTTCTATCAAGATATCTTACAAGGCGTTCGGACAGATCCGACTACGC
stp_48_1: GCAACTCGTTTACAAACTCCCGCAGCCCTCCTAAGCGACAGGCGACAGGCGACTACGC
stp_48_2: GGACGGCTTTCACTAGTACTTTAATTAAAGCTCCTCGAACTTCCGGTGAAGATCCGACTACGC
stp_48_3: CAGCACAGTTCTTTCGAGTGCGAATTCTTACAACTTCCGGTGAAGATCCGACTACGC
stp_48_4: ACCACCGAGTTCCATTAAACATAACCGATATATTCTCACTTGCTGTGCTGGAAGATCCGACTACGC
stp_48_5: GGACAAGGTTGAAACCGAACTAAAACACTCATCTTGGAACTTCCGGTGAAGATCCGACTACGC
stp_48_6: TGTCTGGCTTTGTGAATTTCATCAAACGTATACTTAAAGCTTGTCCGGAAGATCCGACTACGC
stp_95_1: GCACCGCTTTAAGCTATTTCTGCTTAATAGAATCCGACAGGCGACAGGCGACTACGC
stp_95_2: GTCATGGCTTAATCTAGGATAAAAACCAAAATATACTCAGCTGAACTCCTCGAGAGATCCGACTACGC
stp_95_3: GTTGGCACTTTCTGTAATGTAATTCTTAAACACAAACACATGCTCAAGATCCGACTACGC
stp_95_4: GTCTGACTTTGAAGCTCACTCAATATTACCCCGTTGTTCCAAGATCCGACTACGC
stp_95_5: GACAGCATTAAATACGACTTGGAATATTGGTTCTCCATCGAAGATCCGACTACGC
stp_95_6: GGATGCCTGTGTTTTCTGCAAAGCATTTCCCGGACAGGAGGAAGATCCGACTACGC
stp_159_1: GATGGGCTTTTCTGGGCTTCACACCGAGAGATTATCTTGGCTATCGATCCGACTACGC
stp_159_2: GGCAACCGTTTAAATTACCTGGAAGCCTACCTGCCGAACTTCCGGTGAAGATCCGACTACGC
stp_159_3: AGGACGCTTGGAAATTACCTGGAAGCCTACCTGCCGAACTTCCGGTGAAGATCCGACTACGC
stp_159_4: TGCCTCTTGGTAAATATAAGATACCTCTTATCGGTCCTGCTCCTCAGATCCGACTACGC
stp_159_5: GGCACCACTGCGATGTAGTGTTTAGGGATACGCGGAGGCGTGTCGGCGGAAGATCCGACTACGC
stp_159_6: GGCACCACTGCGATGTAGTGTTTAGGGATACGCGGAGGCGTGTCGGCGGAAGATCCGACTACGC
stp_208_1: GGACCGTGTTTTCGGAACCTGAGTCATTCCTCCACGCAATCCCGGACTGTAAGATCCGACTACGC
stp_208_2: TGTGTCGGTTTCTGAAATTTCTATCAAGATATCTTACAAGGCGTTCGGACAGATCCGACTACGC
stp_208_3: GTGGCTCGTTTCACTGGGAGTTCTACCGGACGTCCTTCAAGATCCGACTACGC
stp_208_4: CATGCGCTTGGCAAGTCAGCTGTGTCATAGTTCCTTACGAGATCCGACTACGC
stp_208_5: CAGGGTACCTTTCAATATCAAGATGAGGTCCCTTACGAGATCCGACTACGC
stp_208_6: GCAACAGTTGCTGAAACTCCCGGACAGGAAATAGAATGAATGCAACTGGGACAGATCCGACTACGC
stp_208_7: GATGGCGCTTTTCTGGGCTTCACACCGAGAGATTATCTTGGCTATCGATCCGACTACGC
stp_208_8: GATGGCGCTTTTCTGGGCTTCACACCGAGAGATTATCTTGGCTATCGATCCGACTACGC
stp_208_9: GATGGCGCTTTTCTGGGCTTCACACCGAGAGATTATCTTGGCTATCGATCCGACTACGC
stp_208_10: GATGGCGCTTTTCTGGGCTTCACACCGAGAGATTATCTTGGCTATCGATCCGACTACGC
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stp_144_6: GTCCAAACTTACAAGAATAAGACTCCTTATTACGTAAAGGTGGCACTCCAAGATCCGACTACGC
stp_31_1: CAGCTCACTTTCATTTTCCGTAACACTGAGTTCAAAGGAACGTCCGTCCGAAGATCCGACTACGC
stp_31_2: GTGTCACCTTGAGTCTGCGGATCAAGCCGCTACAAGTACAGGTGCCCAAGATCCGACTACGC
stp_31_4: GTGGCACACCTTCCATACCGACCTGCTCCATGACTAACAGCAATGCGAAGATCCGACTACGC
stp_31_5: GCAATTGGCTTAAAGCTGCTCAAATGCAAGAAGATCCGACTACGC
stp_176_1: GATGTCCTTCCGCGTATCGGGGTTTGCCGTCAAGGACGATCCGACTACGC
stp_176_2: GCTCTGCTTCAAAATAACAGGAAGGCGCTTAATATAGAAGCCCGTCCGCAAGATCCGACTACGC
stp_176_3: GGACGGACTTAACTAAAAGATCTCCAAAAAAAAGGCTTTTGCGGTGGACAAGATCCGACTACGC
stp_176_4: GTGTCCAATTGGATCGTCGGGTAAGCAACGGCTAAAGTACAGGTGCCCAAGATCCGACTACGC
stp_176_5: GTGGCACCTTCGGAAAGATTCGCGACCTGCTCCATGACGTACGACGTCCGACTACGC
stp_176_6: GCAACGGCTTAAGTTAAAGACGTGTTCTTAAACAGAAGAATCCCGCAAGATCCGACTACGC
stp_63_1: CAGCCGAGTTCTCTCAAACAACGCCGCTGGGTACGACATGACTACGC
stp_63_2: AGGAGGCTTCTCAAGGTTGTTTCACTATATAGAAGCCCGTCCGCAAGATCCGACTACGC
stp_63_3: CATCGTCCCTTAAAGGGGGAAAGCTGCTCATTAAAGAGACGTGTTCTTAAACAGAAGAATCCCGCAAGATCCGACTACGC
stp_63_4: GTGGACCCGTATGAAATAGTGACGGAAATTATTCATGTCACCAATGCCTAAGGACGATCCGACTACGC
stp_63_5: CATGTCGCTTACACCTTTAAGCCCAAGAACTTTTTATGATGACCCCCGAAAGGCGTCTGCCGAAGATCCGACTACGC
stp_63_6: TGATGCCTCTTCAAGGTTGTTTCACTATATAGAAGCCCGTCCGCAAGATCCGACTACGC
stp_191_1: GCAAGCGGTTAAGGATTAAGAAACTGCTTAATATAGAAGCCCGTCCGCAAGATCCGACTACGC
stp_191_2: GAAGGTCGTTACATAAAAACAGCCATATTATTTGAGATTACCCTCGTGGAAAGATCCGACTACGC
stp_191_3: GTAAGGCATTTCATTAAAAGAACCACCAAGTTAAATCCCTGCTTACGAGATCCGACTACGC
stp_191_4: GATGAGCGATTCTCCGGCTATGCAAGGACGACCGTCCGCAAGATCCGACTACGC
stp_191_5: GTGGACCCGTATGAAATAGTGACGGAAATTATTCATGTCACCAATGCCTAAGGACGATCCGACTACGC
stp_191_6: CCAGGCGGCTTCAGAAATAGCAATAGCAAGGAATACGAAGTACAGGATCCGACTACGC
stp_mid_complex_1_1: GCAACGGCTTCTTTTGCAATCCTGAATCTGAACATGACGGGCAAGATCCGACTACGC
stp_mid_complex_1_2: GCTTGACCCTTTTGCAATCCTGAATCTGAACATGACGGGCAAGATCCGACTACGC
stp_mid_complex_2_1: GATGAGCGATTCTCCGGCTATGCAAGGACGACCGTCCGCAAGATCCGACTACGC
stp_mid_complex_2_2: GACCGAGTTCTTCAATATTGCCCTAATAACGAAAGGCGTCTGCCGAAGATCCGACTACGC
stp_mid_complex_3_1: GATGAGCGATTCTCCGGCTATGCAAGGACGACCGTCCGCAAGATCCGACTACGC
stp_mid_complex_3_2: GCTGGACGTTTTTGCTGFAATACGACAGGATGTAATATATAGAAGCCGCTTCTTCAAGGACGATCCGACTACGC
stp_mid_complex_4_1: GCTGGACGTTTTTGCTGFAATACGACAGGATGTAATATATAGAAGCCGCTTCTTCAAGGACGATCCGACTACGC
stp_mid_complex_4_2: GTACTCCGTTTCGCTTCTCAATATTGCCCTAATAACGAAAGGCGTCTGCCGAAGATCCGACTACGC
stp_mid_complex_5_1: GCTGGACGTTTTTGCTGFAATACGACAGGATGTAATATATAGAAGCCGCTTCTTCAAGGACGATCCGACTACGC
stp_mid_complex_5_2: GCTGGACGTTTTTGCTGFAATACGACAGGATGTAATATATAGAAGCCGCTTCTTCAAGGACGATCCGACTACGC
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

stp_mid_complex_6_1: GCCTGACGTTGAAATGAGCCACCTAAGGCAAATGCCACTACGTAAGCGAGATCGCAGACTACGC

stp_mid_complex_6_2: GCCTACGGTCGAAAGGCAACCAGAAATCAACCACCATTGGCTCTAGCGAGATCGCAGACTACGC

Seed adapter tile sequences:

AD1SEs6bp_1:: AGGGATAGCAAGCCCACAACACGTGAGGACACTTGAGGGCTGACTCC
AD1_2SEs6bp_3:: TGTCCTCAGCTGCTGGATGCGGATCTACGACACCTTCAAG
AD1_2SEs6bp_5:: CGCTGACTTTGCTGTAGGCGATCAGCATCCAGATAGGAACCCATGTAC
AD2SEs6bp_2:: CCAGACGAGTCGAGAGTCAGCGACTACGC
AD3SEs6bp_1:: GAATTGCAGATAATAAGTGACCTTGCTGTACCGTGAGATGGAGT
AD3_4SEs6bp_3:: ACAGCAAGTGTCACCCGACGGTGCGACTAGCGACATCGACCGGT
AD4SEs6bp_5:: CCACAACCTGTCGCTAGTGCCACTGCGCTTTTTCAGGTTGAAA
AD3_4SEs6bp_2:: CCAGACACTCCATCGGTTTGAGGCTACCTC
AD5SEs6bp_1:: ACCCTCAGCAGGAAACGGATACGGCAACACCGGAGAGCTACG
AD5_6SEs6bp_3:: GTGCGCTGCTGCTAGTCAGCTACGACGTCTCAACTCAGG
AD6SEs6bp_5:: GCTCTGCCCTGAGATCAGCTACGACGATCCGACACG
AD5_6SEs6bp_2:: CCAGAGCTGGCTGAGACGACTCAGTCACACG
AD7SEs6bp_1:: TGATCATCGCCTGATCAACGGTACGAGATGCGAAGCAGAGTG
AD7_8SEs6bp_3:: TCTCGTACCGTTGCCAGTAGACTAGCGACTCCGCTTCGCA
AD8SEs6bp_5:: GTACCGCTACCGCTGCTGCTAGGTCTACTGCGGAAATTCGTGTCGAAATC
AD7_8SEs6bp_2:: CCAGACACCTGCTGCTGACAGTGACTACCCG
AD9SEs6bp_1:: CATTGACGATAGGAGCTATGCGCTATCGCTAGGACCTCTTG
AD9_10SEs6bp_3:: CATAGCATACGGTGTGCTCCAGTCTGCTTGGTCTAGAGGC
AD10SEs6bp_5:: CCACGACTCTCTGTAGGACGACTGACGACACGGTGACAGGAA
AD9_10SEs6bp_2:: CCAGACAGAGGGAGCTAGAGGCTAGCTGCGAGACTCAGACTG
AD11SEs6bp_1:: GAATACCACATTCAACACCGATGAGGATCAGCGGACACTCGACAGCT
AD11_12SEs6bp_3:: GATCCTACCCGTCAGCGAGGGCTGCGAGCCTGTTAGTGCCGT
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

AD12Se6bp_5: GCGGACTGACAGGCTCGCACCTCCTTGTTAATGCAGATACATAA
AD11_12Se6bp_2: CCAGAC CAGTGTGCAGTCCGC TACCTC

Amino modified DNA for coating seeds with PEG:

FLIP seed PEG-attachment strand: /5AmMC6T/AAGCGTAGTCGGATCTC

Labeling strand sequences:

Labeling_strand_ATTO488: /5ATTO488N/TTC TAT CCA CCT CAC CA
Labeling_strand_ATTO647: /5ATTO647N/AAC TAT CCA CCT CAC CA
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

### Fluorescence attachment strand sequences:

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<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tr>
<td>Unused_m13mp18_01_OLS</td>
<td>AAAATCTTACCAGTATAAAAGCCAACCTTTTTGGTGAGGTGGATAG</td>
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<td>Unused_m13mp18_02_OLS</td>
<td>GCCTGTTTAGTATCATATGCCTTTTTGTAGGTGGATAG</td>
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<td>Unused_m13mp18_03_OLS</td>
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<td>Unused_m13mp18_05_OLS</td>
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<td>Unused_m13mp18_06_OLS</td>
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DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

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Unused_m13mp18_30_OLS  AAGGAGCAGGAATTCATCATATACTCTTTTGGTGGAGGTGGATAG
Unused_m13mp18_31_OLS  CATTTTGCGGAACAAAGAAACCACCTTTTGGTGGAGGTGGATAG
Unused_m13mp18_32_OLS  TAATTTAAAAAGTTTGAGTAACATTTTTTGGTGGAGGTGGATAG
Unused_m13mp18_33_OLS  GTATTAAATCTCTGCCCCGAACGTTTTTTGGTGGAGGTGGATAG
Unused_m13mp18_34_OLS  TAGACTTTACAACAAATTCGACAACCTTTTGGTGGAGGTGGATAG
Unused_m13mp18_35_OLS  ATAATACATTTGAGATTTTAGAAGTATTTTTGGTGGAGGTGGATAG
Unused_m13mp18_36_OLS  CCACTAATAGAAGAGCCGTCATTTTGTGGAGGTGGATAG
Unused_m13mp18_37_OLS  ATCTCTAAAAATCTCTTTAGGACAACCTTTTGGTGGAGGTGGATAG
Unused_m13mp18_38_OLS  ACTGATAGCCCTAAAACATCGCCATTTTGGTGGAGGTGGATAG
Unused_m13mp18_39_OLS  GAATGGCTATTAGTCTTTAATGCGCTTTTTGGTGGAGGTGGATAG
Unused_m13mp18_40_OLS  AGAATACGTGGCAAGACAAATTTTTTGGTGGAGGTGGATAG
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Unused_m13mp18_49_OLS  TGATTAGTAATAACATCCTGCTTTTTTGGTGGAGGTGGATAG
Unused_m13mp18_50_OLS  AATAATACGTTGTAACATCTCTTTTGTGGAGGTGGATAG
Unused_m13mp18_51_OLS  CCGAGTAAAAGAGTGTACGGCTTTTTTGGTGGAGGTGGATAG
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Unused_m13mp18_54_OLS  AACAGGAGGCGGATTAAAGGGATTTTTTTTGGTGGAGGTGGATAG
Unused_m13mp18_55_OLS  TCCTCGTTAGAATCATCAGAGCGGGAGCTTTTTTGGTGGAGGTGGATAG
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Unused_m13mp18_56_OLS  GCTTTGACGAGCAGTATAACGTTGCTTTTTTGTTGAGGTGATAG
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Unused_m13mp18_59_OLS  TGGAAGTGTCGGTACGGTCAGCTGTCGTTTTTGTTGAGGTGATAG
Unused_m13mp18_60_OLS  AAGCGAAAGGAACCGGGCGCTAGGCTTTTTGTTGAGGTGATAG
Unused_m13mp18_61_OLS  CGAAGCTGCGGGAATAAGGGAAATTTTGTTGAGGTGATAG
Unused_m13mp18_62_OLS  GATTTAGACCTTGAGCGAAAGCCTTTTTGTTGAGGTGATAG
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Unused_m13mp18_64_OLS  TTTTGGGCTGAGTGTCACCTGAAAGGTTTTTGTTGAGGTGATAG
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Unused_m13mp18_72_OLS  CTGGTTTGCACGGAGGCGAAATTTTTTTGTTGAGGTGATAG
Unused_m13mp18_73_OLS  TGAAGAGGTGTCGACAGAGCGGCTTCATTTTTGTTGAGGTGATAG
Unused_m13mp18_74_OLS  AGCTGATTGCGCTTCACCCGGGGTTTTTTGGTTGAGGTGATAG
Unused_m13mp18_75_OLS  TTTCTTTTACAGTGACGGCCTTTTTTTGTTGAGGTGATAG
Unused_m13mp18_76_OLS  GTTGGGTATTTGCGGAGCAGTTTTTTGGTTGAGGTGATAG
Unused_m13mp18_77_OLS  GAATCGGCAACCGCGGGAGAGTTTTTTGTTGAGGTGATAG
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Unused_m13mp18_79_OLS  TGCGCTACGCGCCGCTTCCAGTCTTTTTGTTGAGGTGATAG
Unused_m13mp18_80_OLS  GAGTGAGCTAATCAGAATTTGCTTTTTTGGTTGAGGTGATAG
Unused_m13mp18_81_OLS  TAAAGTGTAAGCTGCGGCTTGCTATTTTTTGTTGAGGTGATAG
Unused_m13mp18_82_OLS  TTTCCACATAACAGACGGGAGGTTTTTTGTTGAGGTGATAG
Unused_m13mp18_83_OLS  CTGTGGAAATTTGTATCCGACCTACCTTTTTTTGTTGAGGTGATAG

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DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Unused_m13mp18_84_OLS  ATTCGTAATCATGGTCTAGGCTTTTTTTTTTGTAGGATAG
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Unused_m13mp18_87_OLS  ACGAGCTGTAAACACGGCAGCTTTTTTTGTAGGATAG
Unused_m13mp18_88_OLS  TTTGGTAAACGGGCTTCTTTTTTTGTAGGATAG
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Unused_m13mp18_90_OLS  TTTGGTAAACGGGCTTCTTTTTTTGTAGGATAG
Unused_m13mp18_91_OLS  CTGTGGGGAAGGGCATCGGTCTTTTTTTGTAGGATAG
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Unused_m13mp18_94_OLS  GACGACGAGTATCGGCTTTTTTTTTTTGTAGGATAG
Unused_m13mp18_95_OLS  GACGACGAGTATCGGCTTTTTTTTTTTGTAGGATAG
Unused_m13mp18_96_OLS  GACGACGAGTATCGGCTTTTTTTTTTTGTAGGATAG

Biotin attachment linker strand sequences for Nanotube Seed:

Biotin_rightside_01: CTATTATTCTGAAAACATTTTTTCATCGTACTCCT
Biotin_rightside_02: CAGGAGGTTGAGGCAATTTTTTCATCGTACTCCT
Biotin_rightside_03: ATCAAGTTTGCCTTTTTTTCATCGTACTCCT
Biotin_rightside_04: GGTTTACCAGCCAATTTTTTCATCGTACTCCT
Biotin_rightside_05: GACGACGAGTATCGGCTTTTTTTTTTTGTAGGATAG
Biotin_rightside_06: AAACGATTTTTTTTTCATCGTACTCCT

Extended Biotin attachment linker strand sequences for Nanotube Seed:

Extended Biotin_rightside_01: CTATTATTCTGAAAACATTTTTTTTTTTTTTTTTTTTTTTTTTTTCATCGTACTCCT
Extended Biotin_rightside_02: CAGGAGGTTGAGGCAATTTTTTTTTTTTTTTTTTTTTTTTTTTTCATCGTACTCCT
Extended Biotin_rightside_03: ATCAAGTTTGCCTTTTTTTCATCGTACTCCT
Extended Biotin_rightside_04: GGTTTACCAGCCAATTTTTTTTTTTTTTTTTTTTTTTTTTTTCATCGTACTCCT
Extended Biotin_rightside_05: GACGACGAGTATCGGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTCATCGTACTCCT
Extended Biotin_rightside_06: AAACGATTTTTTTTTCATCGTACTCCT
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Sequences of the additional 30 biotin attachment linker strand on extra M13:

Biotin Unused_m13mp18_36  CAACATAAGATTAGAGCCGTCAATTTTTACATCGTCATCCT
Biotin Unused_m13mp18_37  TATCTAAATATCTTTAGGAGCCTTTTTACATCGTCATCCT
Biotin Unused_m13mp18_38  ACTGATAGCCCTAAACATCGCCATTTTTACATCGTCATCCT
Biotin Unused_m13mp18_39  GAATGGCTATTAGTTTTAATGCGCTTTTTACATCGTCATCCT
Biotin Unused_m13mp18_40  AGAATACGTGGCACAGACAATATTTTTACATCGTCATCCT
Biotin Unused_m13mp18_41  ATAGAACCTTCTGACCAAGCGTGGTTTTACATCGTCATCCT
Biotin Unused_m13mp18_42  ATAAAAGGGACATTCTGGCCAACAGTTTTACATCGTCATCCT
Biotin Unused_m13mp18_43  GCAGATTCAACCAGTCACAGCCAGTTTTACATCGTCATCCT
Biotin Unused_m13mp18_44  ATCGTCGAAATTTGATTATTATTTTACATCGTCATCCT
Biotin Unused_m13mp18_45  ATGGAATACTACATTTTGACGCTTTTTACATCGTCATCCT
Biotin Unused_m13mp18_46  CCAGCCATTGCAACAGAAAGCCCTTTTTACATCGTCATCCT
Biotin Unused_m13mp18_47  CTGGTAATATCCAGAACAATAATATTTTACATCGTCATCCT
Biotin Unused_m13mp18_48  GTAGAAGAAGTCAAATACGTGGCTTTTTACATCGTCATCCT
Biotin Unused_m13mp18_49  TGATTAGTAATACATCACTTGCTTTTTACATCGTCATCCT
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Biotin Unused_m13mp18_61  CGAACGTGGCAGAAAAGGAAAGGAATTTTTACATCGTCATCCT
Biotin Unused_m13mp18_62  GATTTAGAGCTTAGCGGCCGGAAGCCTTTTTACATCGTCATCCT
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

Biotin Unused_m13mp18_63: TAAATCGGAACCTAAAGGAGCCCTTTTCACATCGTCACTCCT
Biotin Unused_m13mp18_64: TTTTTGGGTCGAGGTGCCGTAAAGCTTTTCACATCGTCACTCCT
Biotin Unused_m13mp18_65: TACGTGAACCACACCACAAATCAAGTTTTCACATCGTCACTCCT

Universal biotin attachment strand sequences:

biotin modified DNA strand: /5BiosG/AGGAGTGACGATGTG

Mike Pacella’s code, unmodified:

```python
import numpy as np
import matplotlib.pyplot as plt
from skimage.transform import (hough_line, hough_line_peaks,
                               probabilistic_hough_line)
from skimage.feature import canny
from skimage import data
from skimage import io
from skimage.morphology import closing, disk
from skimage.morphology import skeletonize
from skimage.measure import label, regionprops
from skimage.filters import threshold_otsu, threshold_local, rank
import sys
import os
import matplotlib.pyplot as plt
import math
from matplotlib import cm
from matplotlib import path
from skimage import img_as_uint
from scipy import ndimage
from scipy.spatial import distance
from scipy import ndimage as ndi
import Tkinter, tkFileDialog

#modifying the joining detection script to measure the angle of Sisi's nanotubes relative to the x-axis of her images

#constants

#constants
tube_width = 5.0
length_cutoff = 3.0
eccentricity_cutoff = 0.5
end_to_end_distance_cutoff = 10.0

def dotproduct(v1, v2):
    return sum((a*b) for a, b in zip(v1, v2))

def length(v):
    return math.sqrt(dotproduct(v, v))

def angle(v1, v2):
    # return math.acos(abs(dotproduct(v1, v2) )/ (length(v1) * length(v2)))
    return math.acos(dotproduct(v1, v2)/ (length(v1) * length(v2)))

def line_length(line):
    p0, p1 = line
    a = np.array((p0[0],p0[1]))
    b = np.array((p1[0],p1[1]))
    dist = np.linalg.norm(a-b)
    #print dist
    return dist

def make_endpoints_mask(filled_binary_image):
```
#function to determine the endpoints of a nanotube identified via edge
detection/morphological filling  
#need to find all endpoint candidates and find the pair separated by the longest path

#first skeletonize the filled binary image (must be a binary int image)
filled_binary_image = filled_binary_image.astype(int)
skeleton = skeletonize(filled_binary_image)
skeleton = skeleton.astype(int)

#now we make a kernel to compute the endpoints of the skeletonized image
kernel = np.uint8([[1, 1, 1], [1, 10, 1], [1, 1, 1]])

#now we convolve the kernel with the skeletonized image
convolved_skeleton = ndimage.convolve(skeleton, kernel, mode='constant', cval = 1)

#now produce an output mask with only pixels with value 11, these are the endpoints
endpoint_mask = np.zeros_like(convolved_skeleton)
endpoint_mask[np.where(convolved_skeleton == 11)] = 1

return endpoint_mask

def endpoints(region_coords, endpoint_mask):
    #using a previously generated endpoint mask to find the endpoints for a particular tube
    #this will return a pair of tubles with the x,y coordinates of the two endpoints
    endpoints_labelled = label(endpoint_mask)
    potential_endpoints = []
    for endpoint in regionprops(endpoints_labelled):
        if any(i in region_coords for i in endpoint.coords.tolist()):
            potential_endpoints.append(endpoint.centroid)

    #now we will find the pair of potential endpoints with the maximal separation distance, those
    #are the true endpoints
    if len(potential_endpoints) <= 1:
        return None

    pairwise_distances = distance.cdist(potential_endpoints, potential_endpoints, 'euclidean')
    indices_of_max_distance = unravel_index(pairwise_distances.argmax(),
                                           pairwise_distances.shape)

    endpoint1 = potential_endpoints[indices_of_max_distance[0]]
    endpoint2 = potential_endpoints[indices_of_max_distance[1]]
    print endpoint1
    print endpoint2
    endpoints = [endpoint1, endpoint2]
    return endpoints

def are_joined(endpoint1, endpoint2):
    #given two endpoints calculate the distance between them and return True or False for
    #whether they meet the joining criteria
    cutoff = 5.0
    distance = distance(endpoint1,endpoint2)
    if distance <= cutoff:
        return True
    else:
        return False

def calc_distance(endpoint1, endpoint2):
    #simple distance calculation
    distance_squared = (endpoint1[0]-endpoint2[0]) * (endpoint1[0]-endpoint2[0]) +
                       (endpoint1[1]-endpoint2[1]) * (endpoint1[1]-endpoint2[1])
    distance = math.sqrt(distance_squared)

    return distance

# Line finding using the Probabilistic Hough Transform
tube_lengths = []
tube_angles = []
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

```
i=0
cy3_file_list = os.listdir('script_images')
'''
root = Tkinter.Tk()
root.withdraw()

file_paths = tkFileDialog.askopenfilenames()
cy3_file_list = list(file_paths)'''

for i in range(len(cy3_file_list)):
    cy3_file = cy3_file_list[i]
    print "cy3 filename is "+str(cy3_file)
    image_unthresholded = io.imread(cy3_file)
    #thresh = threshold_otsu(image_unthresholded)
    #image = image_unthresholded>thresh

    block_size = 15
    #image = threshold_local(image_unthresholded, block_size, offset=10)
    #image_647 = threshold_local(image_647_unthresholded, block_size, offset=10)

    radius = 4
    #thresholding both files (getting rid of this because it should not be necessary!)
    #image = rank.otsu(image_unthresholded, selem)
    #image_647 = rank.otsu(image_647_unthresholded, selem)

    image = image_unthresholded

    #performing edge detection and morphological filling
    edges_open = canny(image, 2, 1, 50) #originally 2,1,500, where first number is the width
    #edges_open = canny(image, 2) #originally 2,1,25
    selem = disk(radius) #creates disks of a given pixel-width radius that fill in gaps in
    the outline
    edges = closing(edges_open, selem)
    fill_tubes = ndi.binary_fill_holes(edges)
    io.imsave(cy3_file+"fill_tubes.png", img_as_uint(fill_tubes))#

    cy3_endpoint_mask = make_endpoints_mask(fill_tubes)

    #label image
    label_image = label(fill_tubes)

    print "detecting nanotube angles...."
    print len(regionprops(label_image))
    for region in regionprops(label_image):
        if region.area/tube_width >= length_cutoff and region.eccentricity >=
            eccentricity_cutoff:
            if region.eccentricity >= eccentricity_cutoff:
                region_coords = region.coords.tolist()
                region_endpoints = endpoints(region.coords, cy3_endpoint_mask)
                if region_endpoints == None:
                    continue
                endpoint_to_endpoint_vector = np.subtract(region_endpoints[0],
                region_endpoints[1])
                endpoint_to_endpoint_distance = np.linalg.norm(endpoint_to_endpoint_vector)
                x_axis_vector = np.array([0, 1])
                angle_with_x_axis = angle(endpoint_to_endpoint_vector, x_axis_vector)
                angle_with_x_axis *= 180.0/math.pi
                if angle_with_x_axis > 90:
                    angle_with_x_axis = abs(angle_with_x_axis - 180)
                print 'angle with x axis is: ', angle_with_x_axis
tube_angles.append(angle_with_x_axis)
```
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

plt.hist(tube_angles, bins = 90, range = (0,90))
plt.xlabel('Angle in Degrees')
plt.title('Distribution of Angles for All Tubes at 0.16 mL/min')
plt.show()

print "printing angles"
f1=open('angles.dat','w+')
for angle in tube_angles:
    print >>f1, angle
f1.close()

Mike Pacella’s code, modified:

```python
import numpy as np
import matplotlib.pyplot as plt
from skimage.transform import (hough_line, hough_line_peaks,
                               probabilistic_hough_line)
from skimage.feature import canny
from skimage import io
from skimage.morphology import closing, disk
from skimage.morphology import skeletonize
from skimage.measure import label, regionprops
from skimage.filters import threshold_otsu, threshold_local, rank
import sys
import os
import matplotlib.pyplot as plt
import math
from matplotlib import cm
from matplotlib import path
from skimage import img_as_uint
from scipy import ndimage as ndi
from scipy.stats import halfnorm
from numpy import unravel_index
import Tkinter, tkFileDialog

#modifying the joining detection script to measure the angle of Sisi's nanotubes relative to the x-axis of her images

#constants
tube_width = 5.0
length_cutoff = 3.0
eccentricity_cutoff = 0.5
end_to_end_distance_cutoff = 10.0

def dotproduct(v1, v2):
    return sum((a*b) for a, b in zip(v1, v2))

def length(v):
    return math.sqrt(dotproduct(v, v))

def angle(v1, v2):
    return math.acos(dotproduct(v1, v2)/ (length(v1) * length(v2)))

def line_length(line):
    p0, p1 = line
    a = np.array((p0[0],p0[1]))
    b = np.array((p1[0],p1[1]))
    dist = np.linalg.norm(a-b)
    return dist
```

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def make_endpoints_mask(filled_binary_image):
    # Function to determine the endpoints of a nanotube identified via edge
detection/morphological filling
    # Need to find all endpoint candidates and find the pair separated by the longest path
    filled_binary_image = filled_binary_image.astype(int)
    skeleton = skeletonize(filled_binary_image)
    skeleton = skeleton.astype(int)
    kernel = np.uint8([[1, 1, 1], [1, 10, 1], [1, 1, 1]])
    convolved_skeleton = ndimage.convolve(skeleton, kernel, mode='constant', cval=1)
    endpoint_mask = np.zeros_like(convolved_skeleton)
    endpoint_mask[convolved_skeleton == 11] = 1
    return endpoint_mask

def endpoints(region_coors, endpoint_mask):
    # Using a previously generated endpoint mask to find the endpoints for a particular tube
    # This will return a pair of tuples with the x,y coordinates of the two endpoints
    endpoints_labelled = label(endpoint_mask)
    potential_endpoints = []
    for endpoint in regionprops(endpoints_labelled):
        if any(i in region_coors for i in endpoint.coords.tolist()):
            potential_endpoints.append(endpoint.centroid)
    if len(potential_endpoints) <= 1:
        return None
    pairwise_distances = distance.cdist(potential_endpoints, potential_endpoints, 'euclidean')
    indices_of_max_distance = unravel_index(pairwise_distances.argmax(), pairwise_distances.shape)
    endpoint1 = potential_endpoints[indices_of_max_distance[0]]
    endpoint2 = potential_endpoints[indices_of_max_distance[1]]
    return [endpoint1, endpoint2]

def are_joined(endpoint1, endpoint2):
    # Given two endpoints calculate the distance between them and return True or False for
    # whether they meet the joining criteria
    cutoff = 5.0
    distance = distance(endpoint1, endpoint2)
    if distance <= cutoff:
        return True
    else:
        return False

def calc_distance(endpoint1, endpoint2):
    # Simple distance calculation
    distance_squared = (endpoint1[0] - endpoint2[0]) * (endpoint1[0] - endpoint2[0]) +
    distance = math.sqrt(distance_squared)
    return distance

def line_finding_using_the_probabilistic_hough_transform():
    # Line finding using the Probabilistic Hough Transform
DNA NANOTUBE WINDSOCKS AS FLOW INDICATORS

tube_lengths = []
tube_angles = []
shorter_tube_angles = []
longer_tube_angles = []

i = 0
cy3_file_list = os.listdir('script_images')

'''root = Tkinter.Tk()
root.withdraw()
file_paths = tkFileDialog.askopenfilenames()
cy3_file_list = list(file_paths)'''

for i in range(len(cy3_file_list)):
    cy3_file = cy3_file_list[i]
    print "cy3 filename is "+str(cy3_file)
    image_unthresholded = io.imread(cy3_file)
    #tresh = threshold_otsu(image_unthresholded)
    #image = image_unthresholded>tresh
    block_size = 15
    #image = threshold_local(image_unthresholded, block_size, offset=10)
    #image_647 = threshold_local(image_647_unthresholded, block_size, offset=10)
    radius = 4
    #thresholding both files (getting rid of this because it should not be necessary!)
    #image = rank.otsu(image_unthresholded, selem)
    #image_647 = rank.otsu(image_647_unthresholded, selem)
    image = image_unthresholded
    
    #performing edge detection and morphological filling
    edges_open = canny(image, 2, 1, 50) #originally 2,1,500, where first number is the width
    #edges_open = canny(image, 2) #originally 2,1,25
    selem = disk(radius) #creates disks of a given pixel-width radius that fill in gaps in
    the outline
    edges = closing(edges_open, selem)
    fill_tubes = ndi.binary_fill_holes(edges)
    io.imsave(cy3_file+"fill_tubes.png", img_as_uint(fill_tubes))#, cmap=cm.gray)
cy3_endpoint_mask = make_endpoints_mask(fill_tubes)

    #label image
    label_image = label(fill_tubes)

    print "detecting nanotube angles...."  
    print len(regionprops(label_image))
    for region in regionprops(label_image):
        if region.area/tube_width >= length_cutoff and region.eccentricity >=
        eccentricity_cutoff:
            if region.eccentricity >= eccentricity_cutoff:
                region_coords = region.coords.tolist()
                region_endpoints = endpoints(region_coords, cy3_endpoint_mask)
                if region_endpoints == None:
                    continue
                endpoint_to_endpoint_vector = np.subtract(region_endpoints[0],
                region_endpoints[1])
                endpoint_to_endpoint_distance =
                np.linalg.norm(endpoint_to_endpoint_vector)
                tube_lengths.append(endpoint_to_endpoint_distance)
                x_axis_vector = np.array([0, 1])
                angle_with_x_axis = angle(endpoint_to_endpoint_vector, x_axis_vector)
                angle_with_x_axis *= 180.0/math.pi
if angle_with_x_axis > 90:
    angle_with_x_axis = abs(angle_with_x_axis - 180)
print 'angle with x axis is: ', angle_with_x_axis
tube_angles.append(angle_with_x_axis)

midlength = 21 #midlength = np.percentile(tube_lengths, 50)

# separate nanotubes out by length
for j in range(len(tube_lengths)):
    if tube_lengths[j] <= midlength:
        shorter_tube_angles.append(tube_angles[j])
        print 'angle with x axis is: ', angle_with_x_axis
        tube_angles.append(angle_with_x_axis)
    elif tube_lengths[j] > midlength:
        longer_tube_angles.append(tube_angles[j])

print "median tube length is: ", midlength
print "printing shorter angles"
d1=open('shorter_angles.dat','w+')
for angle in shorter_tube_angles:
    print >>d1, angle
d1.close()

d2=open('longer_angles.dat','w+')
for angle in longer_tube_angles:
    print >>d2, angle
d2.close()

mu1, std1 = halfnorm.fit(shorter_tube_angles)
mu2, std2 = halfnorm.fit(longer_tube_angles)

print "mean and std dev of shorter tubes is : ", mu1, " ", std1
print "mean and std dev of longer tubes is : ", mu2, " ", std2

f = plt.figure(1)
plt.hist(shorter_tube_angles, bins = 90, range = (0,90), normed = True)
x = np.linspace(0, 90, 100)
p1 = halfnorm.pdf(x, mu1, std1)
plt.plot(x, p1, 'k', linewidth = 2)
plt.xlim(0, 90)
plt.xlabel('Angle in Degrees')
plt.title('Normalized Distribution of Angles for
Short to Medium Tubes at 0.16 mL/min')
plt.savefig('normalized short to medium 0.16.png')
f.show()

plt.hist(longer_tube_angles, bins = 90, range = (0,90), normed = True)
p2 = halfnorm.pdf(x, mu2, std2)
plt.plot(x, p2, 'k', linewidth = 2)
plt.xlim(0, 90)
plt.xlabel('Angle in Degrees')
plt.title('Normalized Distribution of Angles for
Medium to Long Tubes at 0.16 mL/min')
plt.savefig('normalized medium to long 0.16.png')
g.show()

raw_input()
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Education: Johns Hopkins University, Baltimore, MD: M.S.E. in Chemical and Biomolecular Engineering, September 2016 to May 2018
Rutgers University, New Brunswick, NJ: B.S. in Chemical and Biochemical Engineering with a second major in Cell Biology and Neuroscience Honors Engineering Program, September 2012 to May 2016
Member of Omega Chi Epsilon – Rutgers Chapter

Skills:
▪ Highly proficient with cell culture techniques (plates, flasks, and bioreactors), and other lab equipment up to a Biosafety Level 2 environment, including micropipettes, pipette guns, centrifuges, autoclaves, vortexers, thermal cyclers, spectrophotometers, pH meters, and fluorescent and time-lapse microscopes
▪ Highly proficient with technical writing and presentation in Microsoft Office, Prezi
▪ Proficient with statistical analysis and simulation in Microsoft Excel, Aspen, MATLAB, and Python
▪ Proficient with image capture and processing in ZEN, ANDOR Solis, and ImageJ
▪ Working knowledge of OriginPro, C++, CAD (Autodesk Inventor), Adobe Photoshop

Research Experience:
Master’s Thesis Research, Johns Hopkins University, October 2016 to May 2018
▪ Studied attachment of DNA nanostructures to HeLa cells and glass-bottom microfluidic devices
▪ Developed a flow model and experimental system for making observations in microfluidic devices
▪ Learned advanced protocols for manipulating biomolecules in a nanotechnology environment

Science Undergraduate Laboratory Internship, PNNL Marine Sciences Laboratory, Summer 2015
▪ Characterized light attenuation and salinity tolerance of *Tetraselmis striata* and *Scenedesmus obliquus* under Michael Huesemann, PhD
▪ Developed a scatter-corrected model for light attenuation in microalgae cultures and demonstrated the effectiveness of selective pressure on salinity tolerance
▪ Learned to gather, interpret, and present scientific data in a professional research environment

Research for Credit, Waksman Institute of Microbiology, Rutgers University, Fall 2014
▪ Studied growth and maintenance of *Nannochloropsis oceanica* under G. Charles Dismukes, PhD
▪ Inoculated and monitored cultures of varying medium, nitrate concentration, carbon source, pH, light intensity, and temperature
▪ Learned to measure biomass density of algal cultures as represented by optical density of chlorophyll, oxygen evolution, and time-lapse microscopy

Volunteered Research, Rutgers University, Fall 2013 to Spring 2014
▪ Studied stem cell induction of human fibroblasts under Rick Cohen, PhD
▪ Learned to reprogram fibroblasts using both lentivirus and electroporation techniques
▪ Learned to prepare cell culture media, feed and passage stem cells, measure optical density of DNA, count cells under a time-lapse microscope, and perform immunostaining

Byrne Seminar, Rutgers University, Fall 2012
▪ Studied effects of protein regulation on gene expression in yeast under Andrew Vershon, PhD
▪ Learned to perform transformation, streakouts, and cloning of yeast