Centrifuges

We have two centrifuges related to DNA origami projects. When centrifuging, the balance is very important

Mini Plate Spinner MPS 1000 by Labnet

This is the centrifuge for DNA planes. There is no setting in the spinning speed

1. Put one plane in. Make sure that the bottom of the wells is facing outside, while the rubber sealing is facing inside
2. Spin the knob so that the other slot can be exposed. Put the second plane in. The second plate should have roughly the similar number of wells occupied (essentially the similar weight)
3. Shut the hood and the centrifuge will start spinning. Hold the centrifuge by hand so that it won't be out of control
4. When it gets very hard to hold, press the open button. Then centrifuge will cut power, the hood will pop open, and the spinning will be held on break
5. Wait until it is not spinning at all, fetch the planes out by using the knob. Check the liquid level in the DNA planes to make sure all are on the bottom of the wells

Centrifuge 5425 by Eppendorf

This is the centrifuge for 1.5 mL and 0.2 mL tubes. When using for 0.2 mL tubes, 1.5 mL tubes without the lid are required so that the small tubes won't fail in the centrifuge

1. Put the tubes in. Make sure they are in balanced position and weight. When needed, use tap water and empty wasted tubes
2. Put the cap of the centrifuge on and spin the central knob so that it is tight. Shut the lid. It should lock on its own. If you need to open the lid, press 'open' button
3. Adjust the speed and time. Normally we use rpm (by 'rpm rcf' button) as the speed unit
    a. 1250 rpm for just top speed a few second can push all the liquid to the bottom of the tubes
    b. 5000 rpm 3 min can be the general setting for filter purification
4. Press 'start stop' button. It will start spinning. Check for abnormal sounds. If sound is strange and serious vibration is detected, press 'start stop' to stop and check for faulty balance or damaged parts
5. Once it's ready, it will stop and open on its own with three time of beeping sounds. If the sounds it too noisy, it can be killed by pressing 'open' button

Agarose Gel Equipment

There are a lot of ways to run the gel. Here is one of the ways. Make sure the operator has more than 3 h on the task of running the gel

Gel Preparation

The base of the gel is 0.5× TBE with 11 mM magnesium and agarose powder. To visualise the DNA, EtBr should be added into the gel. It takes more than 0.5 h to prepare the gel.

1. Wash and dry the 125 mL Erlenmeyer flask. Weight 0.5 g agarose powder (for one piece of 1 % gel). Add the powder into the flask and add TBE buffer to the level of 50 mL. Get the 500 mL Erlenmeyer flask ready for washing the smaller flask once the boiling of the gel is finished
2. Heat the 125 mL flask with the heating pad in the fume hood (adjust the temperature to 400 as the value is not accurate). Add tap water into the 500 mL flask and put it in the fume hood with paper towel under it and by the side of the heating pad
3. Once the mixture in the 125 mL flask is boiling, use thermal resistant glove to move it off the heating pad, on to the paper towel. At the same time, place the 500 mL flask on the heating pad. Adjust the temperature setting to 250
4. Quickly get ready the horizontal dock, the holder for the gel and the 8-bit comb ready for solidification of the gel (in the fume hood). It has to be ready by step 6. This is time sensitive as the gel is cooling
5. Wait until the 125 mL flask is about 80 °C (can hold at the neck by thin gloves for 1 – 2 s), add 5 µL of EtBr original solution (10mg/mL). If the visualisation is not good, add 10 µL or even more. Try not to touch the agarose solution with the pipette tip. Stir the flask with hand (thermal resistant glove) for evenly distributed reddish pigment
6. Wait until the 125 mL flask is about 60 °C (can hold at the neck by thin gloves for 10 s), pour the agarose solution into the holder for the gel (make sure the comb is well-placed). Double check the liquid level is horizontal and even
7. Pour ~75 mL warm water from 500 mL flask into the empty 125 mL flask. Place the 500 mL flask on the paper towel and 125 mL on the heating pad. Adjust the temperature setting to 400. Wait for boiling
8. Once the water boils, use thermal resistant glove to pour the water into the small sink inside the fume hood. After this, use the rest of warm water in the 500 mL flask to rinse the 125 mL flask twice. Use the tube brush if needed. The wastewater goes into the regular sink. After warm water, use tap water to rinse it a few times until it is clean
9. Somewhere during step 8, the gel should be solidified. Check it by blow soft wind or tilt on corner of the horizontal dock. If there is no flow, it is a solid piece. Carefully remove the horizontal dock assembly from the fume hood and place it outside once the electric power parts are ready

Electric Power

During the last few steps of gel preparation, the operator should get the electric part ready

1. Take the running dock out and install the electrodes. There are two sets. Pick the shiny set. Check with the cabled covering to avoid the wrong order
2. Carefully remove the holder for the gel together with the gel and comb from the horizontal dock. This might need some practice since it is very easy to rip the edge of the gel. Do not pull the comb out from the gel yet
3. Place the holder in the running dock out. Make sure the comb is at the side of the negative electrode (since DNA is negatively charged, it will move from negative to positive).
4. Add TBE buffer to the dock. Make sure the buffer goes on both sides and just a little over the level of the gel. Once all the gel is submerged in the buffer, carefully remove the comb.

Sometimes the gel can move as the comb is removed. This is not a big problem. Just use clean gloves to gently push the gel back to its place

5. Mix the sample to be run in the gel with loading buffer. Depending on the concentration of loading buffer, the mixing ratio is different. For example, if use 6× loading buffer, mix 15 µL sample with 3 µL loading buffer. The maximum loading volume should be 20 µL. Prepare a ladder with loading buffer. If the running ladder is available, it can be used directly. Otherwise, the stocking ladder is a concentrated solution. Calculate the amount needed for stocking ladder, loading buffer and DI water so that the final concentration of ladder and loading buffer are all 1×
6. Add ladder and all the samples with loading buffer in the 8 wells. Normally ladder is placed at one of the two sides (either #1 from the left or right). Sometimes it can be really hard to see the place of the well. Try different directions and feel that with the pipette tip. Once the samples are loaded, make sure the blue-purple liquid is not escaping
7. Put the cabled covering on and plug the cables into the power supply. Check the electric current again. The DNA samples should be places near the negative side. If the order is wrong, simple reverse the cables in the power supply
8. Adjust the voltage to 20 V. Test run to see if there are any error codes. Sometimes there can be an error stating no current. This could be a bad electrode. Try to clear the rusty part may solve the issue. If there is nothing wrong, both electrodes in the buffer should be surrounded by tiny bubbles. If not, wait a bit or do the same procedure as if there is no current

Running and Checking the Gel

When have time, rinse all the used equipment and dry them on the paper towel

1. Wait for 10 min at 20 V or until the directing of sample movements is visible. If the direction of moving is wrong, flip the cables in the power supply
2. Add the voltage to 60 V. Let it run for 2 h. If condition permits, check it frequently and add ice packs to keep the temperature low
3. Once the colour bands from the loading dye have pass the half of the gel, it can be taken out for visualisation under UV light
4. When taking the holder for the gel and the gel together from the buffer, make sure it is tilted to one side so that the buffer above the gel can flow back down into the running dock. Use the gloves to hold the lower side so that the gel won't slip down with the buffer. The 'exhausted' buffer can be reused for the next gel
5. Use the Spectroline TE-312S UV Transilluminator to shine UVB from the back side of the gel and holder. The bands should be separated. If not well-separated, the gel can be placed back to the buffer for further running
6. For better visualisation and photos, the operator may remove the gel from the holder. Be really careful in case the gel breaks or fails on the floor. The operator may also open the plastic covering of the transilluminator. Before opening it, UV face shield should be placed
7. Recovering origami from the gel can be referred to the instruction manual of 'Freeze N Squeeze' on BIO-RAD website

8. Clean everything related to the gel. The gel itself and anything in contact with concentration EtBr (gloves, paper towels and sometimes blades) must be disposed into the special trash can for EtBr

Thermal Cycler

It can also be named 'Thermocycler', 'PCR machine' or 'DNA amplifier'. There are 2 BIO-RAD S1000 thermal cycler in our lab. They are almost identical. S1000 has a lot of functions, of which we typically just a small part. It can only take 0.2 mL PCR tubes

General Pre-Setting

There are a lot of previous protocols, which can be used either directly or with some minor modification. Before modification, making a copy is recommended

1. Lid temperature. Set it 5 °C above the maximum tube temperature. This is for avoiding the drying of liquid during the temperature ramps
2. Initial heating. Set it at 75 – 95 °C so that the DNA strands can fully melt for annealing. Not needed for incubations
3. Actual temperature ramp. The logic of the S1000 is a temperature with a duration. The resolution is 0.1 °C and 1 s. For example, 95 °C for 1 min
4. The increment or decrement is realised by 'Go To' function with + or – per cycle. For example, step 2 can be '95 °C for 1 min with -0.1°C per cycle'. Step 3 can be 'Go To step 2 for 500 times'. As a result, we have -0.1°C per 1 min, from 95 °C to 45 °C
    a. There is a temperature per time in the setting. Based on Haorong Chen's experience, it is not as good as temperature per step. Again, explorations are always welcome
5. The ending temperature is normally set as 25 °C, and can go as low as 20 °C. Below that is generally unnecessary. At the very end, hold the temperature at 4 °C forever (0 for duration)

Regular Annealing and Incubation

Prepare mixture in 0.2 mL PCR tubes. Don't add more than 100 µL liquid in a single tube. If necessary, separate into 2 or more tubes. Place them in Block A or B and shut the lid

1. Twist the knob and make sure there is one clicking sound indicating the lid is tight
2. Use ' run' to select the protocol and execute in on Block A or B, depending on the place of the tubes. The volume doesn't matter and can just go with the default (50 µL)
3. Once it starts, use the function button on the left to change the view and see if the temperature ramp is correct. If not, cancel it as early as possible to avoid lost of time and material
4. The time estimated by the machine is in accurate. Better estimation is by running the protocol and know. The actual time needed is always about 1.5 times the time estimated

Special Tricks

Sometimes we need operation between different temperature ramps. Some special tricks can make the tasks a lot easier

1. Go to the next step. In the view of current running protocol, press 'enter' can pop some options out. Press go to the next step can skip the current step. For example, step 2 is 95 °C for 30 min and step 3 is 65 °C for 30 min. When in step 2 for 20 min, it can go directly into step 3 by going to the next step
2. Add holding at certain temperature forever. If adding strands at certain temperature is needed, hold the temperature at that forever and make sure it is holding by the view. Twist the knob in the opposite direction and open the lid. Try to add strands fast as the temperature can drop really quickly when the tube is outside the thermal cycler. Use the 1st trick once the tube is back to the cycler

UVP lamp (model UVGL-25)

The lamp can emit UV light centred around 254 and 366 nm which may be switched by applying short- and long-wavelength settings, respectively. It is used as the source of UVC and UVA. UVB can be found in Spectroline TE-312S UV Transilluminator

Preparation

Use Homemade quartz tube (inner cross-section size: 2 × 4 mm) for holding liquid in place. Unlike a glass tube, the quartz tube is transparent in the UV spectrum, thus suitable for UV irradiation

1. Dilute the sample to 2 nM 20 – 40 μL using MES buffer and keep the solution in quartz tubes
2. If there is any additive to the DNA solution, add it. For example, mix saturated TP1 solution with DNA solution for a final volume ratio of TP1 solution to mixture at 1:10
3. Find the partial cut pipette tip box for 20 μL tips. Attach the quartz tubes to the back side of the covering

UV Irradiation

Place the pipette tip box with tubes at about 1 cm in front of the UV source.

1. The timer could be set by on the AC power or just by phone/computer
2. In order to avoid all other lights, cover the box and the UV source with opaque material'

Bruker Dimension Icon atomic force microscopy (AFM)

AFM is high-resolution scanning force microscopy, which uses a laser beam to detect the deflection of a cantilever with a tip scanning the sample, normally on a flat surface. For the AFM equipped with ScanAsyst-Air or ScanAsyst-Fluid+ probes, Peak-Force tapping mode is normally used for scanning, which can acquire high-quality imaging in air phase without damaging the sample significantly. With the correct setting, the mechanical properties can be acquired as the same time

There are quite some modes available to AFM measurements and a big number of probes for different functions. It's not possible to list everything feasible here. Rather, some typical measurements/calibrations are summaries here. Details need to be referred to the Bruker's help documents, PDF or video training materials

Every day using the AFM, put the date and user's name in the logbook. If anything happens, also log it. Every time after using it, turn off the software if the next user is more than 30 min later. If not sure, ask around. It won't hurt to turn off even if the next user is just 10 min away. At the end of the day, double check the off state of the software and the laser. Sometimes the laser stays on after the software is off. This indicates a faulty communication between the computer and the AFM (through a DSP cable). The related data collecting card was replaced by a good used unit in 2018-2019. It turns out to be better than the previous one. This faulty communication should happen less than once a month. Turn the system completely off on a weekly base (better 5-day base). This should maximise the longevity of the system

Regular Dry AFM

Select ScanAsyst-Air probe and use in air probe holder. It's possible to use air probe in liquid holder, liquid probe in air holder or liquid probe in liquid holder but measure in dry condition. These don't give good results in general. Unless needed, don't use unconventional setting for publication data. The samples have to be fully dried before imaging. Partial dried sample can interfere with the track of laser light and thus not recommended

1. Deposition of origami cylinders and tiles
    a. Dilute origami cylinder, tiles, or cyclized tiles to 0.5 nM with TAEM buffer
    b. Cleave mica surface using scotch tape. Before taping, gently scratch half of the upper circular edge of the mica with the back of a tweezer. Attach the tap. The peeling direction is from the scratched side to the non-scratched side. Double check that the mica surface is the only sticked surface on the tape and the peeling side is well attached. Peel the tape and cover the mica surface with a petri dish. Check the tape to see if an intact round surface is peeled off. If so, there is a freshly cleaved surface. If not, repeat peeling again. Sometimes it can be really hard to get peeling right if focused on a single mica. Try another mica might be beneficial
    c. Pipette 10 μL aliquot onto freshly cleaved mica surface, cover it with a petri dish and start the timer for 5-min incubation at room temperature. Stir the liquid on mica for 2 rounds with hands holding the petri dish
    d. Use compressed air to blow the mica dry, rinse with 80 μL DI water for about 3 s and blow the mica dry again to keep it from contamination by covering it
2. Deposition of polymerised ribbons or other origami structures
    a. Dilute polymerised origami to 2 nM with MES buffer
    b. Pipette 10 μL aliquot onto freshly cleaved mica surface, cover it with a petri dish and start the timer for 5-min incubation at room temperature. Stir the liquid on mica for 2 rounds with hands holding the petri dish
    c. Add 20 μL fixing buffer (TAEM buffer with 2 mM nickel chloride) to the same mica surface, cover and incubate for another 2 min at room temperature. Also stir the liquid for 2 rounds as early as possible so that the ions can spread out
    d. Use compressed air to blow the mica dry, rinse with 80 μL DI water for about 3 s and blow the mica dry again to keep it from contamination by covering it

3. There are other deposition methods with different origami concentration and buffer conditions. For example, 1 nM origami monomer or using TAEM an fixing buffer for polymerised origami structures
4. Use in air Peak-Force tapping mode to measure. Check the probe type in the set up before any scans
    a. Make sure the laser is at the back of the tip of the probe using shadow method. Adjust to get the regional maximum sum value (generally 4.8 V). Careful tune so that the horizontal and vertical difference (absolute value) is less than 0.1 V. Sometimes this is hard. Use the little window on the head can make it easier. For ScanAsyst-Air probe on air holder, the laser dot should be on lower centre of the glass if the horizontal is set as the major axis
    b. By default, the relative heights of optics for the back of the tip, the sample and the tip reflection have defined relationships. The most important one is that the tip reflection is 1000 μm lower than the sample. During engaging, the tip will first move down 800 – 900 μm and slowly finish the rest until it touches the sample surface. The threshold of touching can be adjusted to reflect the sample properties. If unknow, better go with the default
    c. Find a place on the sample where it is free of dust dots in the sample view and can see the tip reflection clearly in the reflection view. The crack on the sample surface can be good for locating the sample but harmful for the tip. Don't scan exactly on a crack. Keep a note of bulk part number on the z values are very important against crushing the tip during finding a good location
    d. Set the engaging parameters. Scan scale: 500 nm, x and y offset: 0 nm for the first scan, Z range ~12 μm. Engage
    e. Once the scan is stable, change Z range to 3 μm, and wait for stable scan. Change the scan scale to 2 μm (type '2u' for any previous units or '2000' if the previous unit is nm). Depending on the DNA origami size, maximum scan scale could be 5 or 10 μm. We usually go with 500 nm, 2u, 5, and 10 if need. 1If 10 μm, then the sample per line should be set to 256 instead of 512. Remember, the resolution of AFM is typically 2 nm. Thus, more than 1 sample per nm is generally not necessary
    f. Sometimes the force curve is not stable. Try 'auto configure' in the force file region first. If not, change peak force amplitude to a bigger value might help. Better change is when the scanning size is 500 nm. After changing peak force amplitude, wait until the scan is stable and click 'auto configure'
    g. Once everything seems reasonable, click 'scanning from the top' or 'scanning from the bottom'. Wait until the frontline hits the other size, hit 'Ctrl + B' for save. If just want a part, hit 'Ctrl + N'. If feels saving is unneeded, skip saving
    h. Use offset or zoom to change the place of scanning if the centres do not match exactly. Avoid a very large x or y offset value
    i. Once everything has been scanned and saved, withdraw and prepare for the next scan. The last scan at any given place can be saved easily by 'capture withdraw'. This function will automatically save and withdraw once the scanning frontline has touched the two boundaries

j. For the new scan, don't make assumptions on the focus. A lot of time, it could be still as required by step c. However, sometimes the mica surface is tilted, and thus a small movement in plane will cause detectable changes in height, which can induce crushing of the probe or very long time of waiting before engagement. Check the sample and reflection views and make necessary adjustments
k. Once all the scan on a certain mica is finished, lift the head of AFM at least 1 mm if the next scan is the same setting. If the next scan is going to be liquid or unknow condition, go with at least 4 mm
l. If the mica is dry, it's fine to leave it inside the AFM hood. It might be good for scan even overnight. However, don't keep a lot of mica disks inside. Take them out frequently

Regular Liquid AFM

Select ScanAsyst-Fluid+ probe and use in liquid probe holder. Avoid drying during the imaging process

1. Deposition of origami structures
    a. Get two pipettes. One for 2 μL sample and the other for 3 μL 25 mM nickel chloride. Suck the liquid aliquots in, respectively. Rest the pipettes on the desk. Due to the small amount, the liquid won't escape the pipette tips
    b. Cleave a mica surface and cover it with a petri dish
    c. The following processes have to be quick. Thus, practices with used pipette tips and tap water on dirty mica surface is very important. Add the sample and nickel chloride onto the mica, start a 30 s timer. Quickly cover the mica with a petri dish and stir to have the liquid cover most of the surface
    d. During the 30 s time, transfer the mica into the AFM hood, on the magnet. Once it is in place, the time is usually up or even late. Add 80 μL of the same buffer as in the sample to the mica
2. This deposition method is adapted from Hao Yan's work. It's subject to be changed as time goes on. For example, we can add Ni^{2+} into the buffer to avoid drastic change in concentration.
3. Use in liquid Peak-Force tapping mode to measure. Check the probe type in the set up before any scans. It's fine to adjust the laser and find the surface in other modes. Remember to change the mode back before the scan
    a. The general procedure of in liquid is similar to in air. The difference is that liquid will case the light to go on a different track and the position where the laser hits can be hardly visible
    b. Align the laser in air will be much easier. Make sure the laser is at the back of the tip of the probe using shadow method. For ScanAsyst-Fluid+ probe in liquid holder, the maximum sum value is 2 – 3 V in air. Sometimes 1.5 V is fine. The shadow might be hard to see, especially the laser dot. It can be big and vague. Careful tune the horizontal and vertical difference. The laser dot should be on upper left of the glass window if the horizontal is set as the major axis

c. Sometimes the horizontal and vertical difference can't be supressed no matter how the knobs are adjusted. This can be solved by placing the probe in a different place on the probe holder
d. For convenience of following measurements, the mica for shadow method should be the mica for deposition or very similar to that. Thus, the height will be the same. Record the Z value when reflection is clearly viewed in tip reflection view. Lift the head up 1 mm so that sample is clearly viewed in tip reflection view
e. Release the head from the locked position. If viewed from the top, the head is going to fall freely a little downwards and to the hood door direction. Push it in the front (from hood door to the back of AFM) so that it won't fall. Life it up (avoid touch the lenses and similar optical parts) and flip it cover
f. Get the mica with > 80 μL liquid ready with the sample deposited. Add 40 μL buffer (same as in the sample) to the lens on the liquid holder (installed on the head). Carefully flip the head back and let it slide back in place. Similar to uninstallation, push its front so that it won't fall too much. Lock it in place
g. The two droplets (80 and 40 μL) should have merged. Due to diffraction index difference between air and water, the track of light will change. Use the check box to compensate the difference of white light and adjust the laser to the right until the sum is big again. It can be really hard to see where the laser dot is. The operations are mostly by experience and understanding of the light
h. If the droplets are separated, try to get a good sum and lower the head. Otherwise add more liquid or redo the whole process
i. Adjust to get the regional maximum sum value (generally 4.2 V with liquid). Careful tune the horizontal and vertical difference (which is very different from dry condition)
j. Carefully lower the head until the tip is around 1.1 mm above the sample. Set the engaging parameters. Scan scale: 500 nm, x and y offset: 0 nm for the first scan, Z range ~12 μm. Try engaging
k. Z range and scan scale changing are all similar to dry AFM. The sample per line should be set to 256 regardless of scanning size. Force curve adjustments are also similar. Just that the peak force amplitude is normally bigger, can start with 50 nm
l. Scanning and saving are the same. Liquid separation after withdrawing happens a lot. Be prepared to add more liquid as scan goes or add a little more at the beginning
m. Once all the scan on a certain mica is finished, lift the head of AFM a little and remove the head. Clean all the liquid on the sample. If need to lower the head, try an air holder with bad probes just for laser reflection. Never leave wet equipment or sample inside the hood for too long, especially when not scanning

Nanoindentation

Available in both air and liquid. The overall procedure is similar to regular measurements with some dedicate calibration on the system and probe's properties

The Z sensor is assumed to be accurate in this section (whose calibration is introduced in the next section). The deflection sensitivity is calibrated based on Z sensor and a very hard surface (Young's

modulus 100 folds of the probe). During deformation, almost all the movement in Z direction is going to be the deflection. This sensitivity is a property of the system, meaning that if the laser is adjusted or the media (air, water) is changed, the value will be different. Based on the experience, if just adjust the laser a little bit, the value is not going to change more than 5 %, which can be assumed identical. With deflection sensitivity, the spring constant is calibrated by thermal tune. This is a property of the probe only. Changing the media and other setting may affect it and causing more than 10 % of changes, but it is fine as long as the method is kept consistent. There is a third parameter, tip radius. This is calibrated separately. It's a property of the probe only. If calibrated in liquid, the tip is abnormally sharp. We better calibrate that in air regardless of air or liquid probe. It's executed by scanning slowly on a rough, sharp sample and using the software to calculate based on the indentation depth. It's more of a contact radius rather than a tip radius. There are other calibrations which we normally don't perform

1. In air
    a. Similar to 'Regular Dry AFM', align the laser and find the sample surface. The sample should be the sapphire sample in the standard sample box. Keep those sample as clean as possible. Sapphire surface might be hard to recognise, just like glass surface. Look for scratches to locate the surface. If the reflection is clear in tip reflection view, it means the head is too low. Rise it up a bit for a clear view in sample
    b. Engage. No need for larger scan size. Once some scans area is available, use offset to find a place without contaminations and go to ramp. Set the trigger threshold to 0.5 V and perform 2 – 3 ramps. Open the files. Correct the baseline and select the indentation region. Find 'Commands > Update sensitivity' to acquire the values and average by calculator. The value should be 60 – 70 nm/V, but currently we have been getting 100 nm/V. The Bruker technician can't find the reason
    c. Open thermal tune window. Enter the sensitivity value and hit 'acquire data' (in air). Then select the region with the peak and fit data. Later, calculate k. It should be 0.35 – 0.4 N/m (marked as 0.4).
    d. Place the sapphire sample back and take the rough, sharp sample out. It has a really good surface so finding the surface is very easy. Make sure adjust the values based on Bruker's document (page 17/35). Need to use extensive mode for more adjustable parameters
    e. Save the image and analysis in the software. For spherical model, tip radius is required. The maximum indentation depth should be the tip radius. Tip radius is a function of indentation depth. Thus, try a few values until they are very similar. Use that value as the calibrated result (new probe < 10 nm)
    f. During measuring, make sure the modulus from spherical and conical models are both turned on. Sample per line is 256 or the software can't calculate so fast. Set the scan rate to 0.2 – 0.4 Hz. Set Peak force frequency to 1 kHz for better result.
    g. Capture a line and find the indentation curves. Export them and make sure the indentation depth is around the tip radius. If not, a just the max force. Few nN is reasonable
2. In liquid

a. Calibrate the tip radius in air first as it's inaccurate in liquid. Wet and dry is not ideal although it's better to calibrate the tip radius after indentation on a hard surface. It's always a trade off
b. Similar to in air but in liquid. The sensitivity is ~ 25 nm/V. Fit a good flat line in finding spring constant in liquid (double check the mode). It should be 0.6 – 0.7 N/m (marked as 0.7).
c. Sometimes in liquid requires larger peak force amplitude. It can go as large as 300 nm. Always auto configure for better force curve. 2 × 2 μm is a good scan against drift in liquid
d. Sometimes drift can be really serious. We haven't figure out a good way to supress it yet

Calibration in general

Every 6 month, the Z sensor needs to be calibrated. The process uses contact mode and a standard sample with pits marked with known depth and spacing

Basically, it requires aligning the sample to the horizontal direction and scan a certain pit. Measure the depth and check for difference. If within 2 %, then the senser is fine. Otherwise, the sensitivity needs to be updated. The height and height senser need to be calibrated together. Generally, height senser is thought to be more accurate

We use open loop calibration in our system. It's a simplified method from the help document. There is a piece of piezoelectric ceramics (short as piezo) that controls the height of the probe holder. Perform the calibration at -50 V, 0 V and 50 V of piezo voltage. By default, it is around -50 V. This means the head is a little low. The piezo retract a little bit so that the probe is not pushing too hard on the surface. This is preferred. By move the set motor, different value can be acquired