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