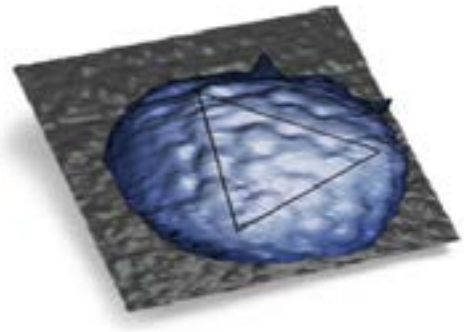
COVALENT METROLOGY





COVALENT ACADEMY Q&A

Episode 8: Applications of Atomic Force Microscopy in the Biosciences

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01

Q: Often time we use poly Ethelene Glycol (PEG) to functionalize Biomaterial and Semiconductor materials' surface; is it possible to image the PEG chains orientation with a change in its molecular weight in a liquid on a substrate?

A: PEG is commonly used in tip functionalization as a linker between the tip and molecule of interest and not a sample to image by itself. It might be challenging to see differences in chain orientation if these differences are subtle or small. There has been one publication (that I know of) out of UC Davis that used our AFM to look at PEG dedrimers. I usually do not discourage people from trying a sample just because it has not already been done. This is one of those cases and I would recommend trying to image it to see what resolution is capable.

Reference:

Riechers, S., Zhong, Q., Yin, N.N., Karsai, A., da Rocha, S.R. and Liu, G.Y. , 2015. High-resolution imaging of polyethylene glycol coated dendrimers via combined atomic force and scanning tunneling microscopy. *Journal of drug delivery , 2015 .*

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02

Q: Has anyone tried to use AFM for DNA sequencing? (central to many of the engineering techniques i have seen is a DNA being pulled out of a pore (can be made via Helium ion beam)) Perhaps AFM can be used as a controlled forcing function to grap and pull the DNA.

A: That has been the dream application from the very beginning of AFM. We have not been able to image DNA molecules at high enough resolution to identify individual base pairs. Some research groups have had reasonable success using a nanopore (see below) but it is still not a straight-forward experiment.

References:

Qamar, S., Williams, P.M. and Lindsay, S.M., 2008. Can an atomic force microscope sequence DNA using a nanopore?. *Biophysical journal , 94* (4), pp.1233-1240.

Kim, Y., Kim, E.S., Lee, Y., Kim, J.H., Shim, B.C., Cho, S.M., Lee, J.S. and Park, J.W., 2014. Reading single DNA with DNA polymerase followed by atomic force microscopy. *Journal of the American Chemical Society , 136* (39), pp.13754-13760.

03

Q: For the data taken on the cancer cells, the image was taken over 90 um (the full scanner range). What tips do you have to collect data over such large scans?

A: Imaging cells in general is challenging since they are soft, tall and may be loosely bound to the substrate. Although it may seem counter intuitive I always recommend using contact mode to image cells. First it is important to firmly attach cells to the substrate (glass coverslip, petri dish, etc.) which may mean pre-treating the substrate with a bioadhesive (collagen, fibronectin, etc.). You also want to use a soft cantilever with a k ~ 0.02 N/m for live cells or k ~ 0.1 N/m for fixed cells. Ideally the cantilevers should have a duller tip with a minimum radius of 20nm to prevent tearing of the membrane surface. If the cells are tall (>10µm in height), which is the case for cancer cells, then you should use a cantilever with a taller tip and an AFM that has a larger Z range. It is also important to image at a slower scan rate. For cell imaging I prefer to look at the scan speed value instead of the scan rate since speed also takes into consideration the scan size. For live cells I recommend a scan speed of 20-30 µm/s. For fixed cells you can go faster using scan speeds of 30-50 µm/s. Finally, you should optimize the setpoint and integral gain to get the best tracking of the surface.

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04

Q: Any new results on Corona?

A: Right now we don't have any new data but I can assure you that there will be some data in the near future.

05

Q: There are a number of bio-tech devices which are used for everything from DNA sequencing to Antibody determination. Have you done work on devices like this and can you give us some general guidelines.

A: I have not done a lot of work in the area of nanodevices. But I can see AFM playing two roles in this field. One as a method to qualify the devices themselves by providing information such as roughness, distribution or density. Another role could be measuring the results. For example, if a particular nanodevice looks at the binding of a molecule (i.e. antibody) to the patterned surface, AFM can be used to image the device before and after the introduction of the antibody to resolve morphological differences. Although AFM may not provide specificity the way fluorescence would to a labeled molecule you will see an increase in size as the antibody binds to its target on the device.

06

Q: Obviously sample preparation is a key ingredient in getting good results. Can you discuss in a little more detail your methodology and how you go about getting these great results.

A: I always begin by saying that AFM can resolve any features, large or small, on the sample surface including contaminants. Therefore, it is very important that samples have been purified and buffers are filtered. Make sure your substrate and cantilever holder are clean. Just keep in mind that when you add your buffer it will pick up all of the dirt and salt crystals present on these surfaces. Next, make sure that you are using a fresh cantilever. In air we have the luxury of re-using the probes until they break or become dull and contaminated. In liquid, however, it is difficult to re-use the probe since they are immediately coated with the buffer and become contaminated with sample over time. There aren't guaranteed methods to clean the probes so it is best to start with a fresh probe with every experiment. Finally, once you start scanning use gentle forces to image. For example in tapping mode decrease the setpoint enough to track the surface well but not too much to damage the surface.

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07

Q: Has anyone used AFM to look at the effects of anti-viral and anti-biotics and the virus/cell level.

A: There are several existing publications looking at virus structure, viral infection of cells and effects of anti-virals on viruses. I showed one example of the binding of catalytic enzymes to the H1N1 influenza virus.

I would recommend reading the recently released application note titled “Advancing Virology Research with High-Resolution AFM Imaging”, available on Asylum Research’s webpage:

https://www.oxinst.com/news/new-application-note-advancing-virology-research-with-high-resolution-afm-imaging/.

08

Q: I see a number of the images that are over large scan areas. Is this the result of having an x-y stage and a z positioner and if so can you discuss some of the pros and cons of this approach versus the x,y,z piezo used by some.

A: We have micrometers on the XY stage which allows us to move the sample/ROI to the area beneath the cantilever but the actual scans are acquired with the XYZ piezos. The maximum XY range varies depending on the AFM but for the Infinity BIO the maximum range is 120 µm.

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