American Society for Investigative Pathology – ASIP.org

MODALITY OF THE MONTH
A MONTHLY METHODOLOGY REVIEW FROM THE ASIP
DIGITAL AND COMPUTATIONAL PATHOLOGY SCIENTIFIC INTEREST GROUP

June 21, 2016

MoM#4: ATOMIC FORCE MICROSCOPY (AFM)
Stanley Cohen, MD

Introduction. The technique of atomic force microscopy (ATF), which can measure forces with sub-nanometer level resolution, was an evolution of scanning tunneling microscopy (STM) that exploits quantum mechanical effects, and for which the inventors received a Nobel Prize in 1986. In STM a bias (voltage difference) between a very fine probe tip and the specimen is created. This can allow electrons to tunnel through the space between them. As the tip is moved across the sample in the X-Y plane, the variation in height and density of states causes changes in current. STM is generally applicable only to electrically conductive samples, and thus is not very useful for the investigation of most biological specimens. A few years after STM, similar probe technology was used to develop AFM. Because AFM samples need not be conducting, it is capable of biological applications as well as those in physics and engineering. Although the field has grown exponentially since then, there has been little interest in or even awareness of the utility of this modality by experimental pathologists. In fact, most publications using AFM to investigate biological materials have involved elegant engineering but relatively unsophisticated biology. The purpose of this MoM is to make the nature of this technology more accessible to pathologists so that it can be better exploited for the study of disease, especially since there are many commercial sources of atomic force microscopes and they are not particularly expensive by current standards.

Mechanism of AFM. AFM is based on the detection of forces acting between a microscopically sharp probe (the AFM tip) and the specimen's surface, and the imaging of those forces. The forces can be due to atomic scale phenomena. Actual resolution is of the order of fractions of a nanometer, three orders of magnitude better than the optical refraction limits, and this is easily attainable for point measurements. However, for multiple data points needed to reconstruct an image, resolution is limited by the number of data points obtained as the tip traverses the specimen, and typically good biological images can have a resolution in the one micron range, although individual measurements are at the nanometer scale. This is due to the limitations of scanning rate and stepping-motor, data acquisition, and computer processing, so it is likely that very high resolution images will become available for direct comparison to confocal super-resolution images of the same or adjacent field after suitable treatment of the specimen.

In essence, the AFM transduces those atomic scale events into detection of tip movement. It does so by measuring the motion conveyed to a flexible cantilever to which the tip is attached. The tip is brought to contact or near contact with the sample surface. As the tip is scanned over the surface the cantilever will be deflected by forces between the probe and surface. The deflection of the cantilever as it scans the specimen surface is usually measured by reflecting laser light off the cantilever and detecting that light by an appropriate sensor. The deflection signal is recorded digitally for computer processing and analysis. Since the specimen is scanned, the signals can be

used to generate an image, by recording the position of the sample with respect to the tip as well as recording the probe signal. As the tip transverses the specimen it moves with the specimen, and so provides topographic, or 3D (z-axis) information about the surface.

The best way of thinking about this process is by way of an analogy. Essentially, the AFM is similar to how a musical recording used to be played before the days of digital CDs. Those of us who still remember (and sometimes play) vinyl records will recall that the components of the player include a cartridge-stylus assembly, tonearm, and rotating platter on which the record sits. The stylus tip and cantilever, which correspond to the AFM tip and cantilever, track the grooves in the record and are vibrated by them. The cartridge transduces the interaction into a measurable event (usually an electrical signal although optical means have also been employed), and the tone arm-rotating platter represents the scanning mechanism. In this analogy, your stereo system takes the place of the AFM computer.

AFM modes of operation. There are several modes in which an AFM can operate:
Contact mode: Here the AFM tip is in actual contact with the specimen surface. In this case, a feedback system is utilized. When the tip comes across a macroscopic bump in the surface, the whole cantilever system is raised and *vice versa* if a trench is encountered. Thus there is a contact "load force" between tip and sample. In this mode, the tip can damage the specimen (scratching) and introduce artifacts. A modification of the contact mode is known as "tapping" which is an intermittent contact mode. In this mode, the tip oscillates up and down rapidly, touching the specimen surface for a short time relative to the actual scanning, thus minimizing contact time between tip and specimen surface.

Force mode: This is not an imaging mode per se, but it can be used to reconstruct an image as will be described below. It is used to measure forces acting between the AFM tip and the surface of interest at a particular point. In this mode, the cantilever, instead of moving in a lateral direction, moves up and down, thus elevating it from the tip or approaching it. When the tip is far from the specimen, the force between them is, in essence, zero. As the tip is brought to the surface of the specimen the measured force increases. When the tip and specimen touch, the AFM operator moves the sample down and tip and sample move together. Next the sample is moved down. The force curve before contact and right after retraction are known respectively as the approach curve and retracting curve. During retraction, the surface may display viscous and elastic properties, which are also measurable. Also measurable is the force required to separate the tip from the specimen surface (adhesion force). Thus a large amount of high resolution information can be obtained about various mechanical properties of the specimen. However, in this mode there is no topographical (z-axis) information.

Although force mode is not intrinsically an image mode, the specimen can be sampled at multiple points and an image reconstructed from the force intensity at these points. For historical reasons, this kind of image is known as a "heat map." By definition, a heat map is a 2 or 3 dimensional plot of the intensity of a variable across a defined region. Since the resulting image is not generated by light and optics, but rather is a computer-generated representation of some other physical

process, the heat map is also sometimes known as a pseudo- or false image, which is misleading since what is seen is a visual representation of actual data.

Force volume mode. Classical force mode provides no intrinsic topographical information, so force volume mode was developed to allow both force measurement and topographical information to be obtained simultaneously. The precise means by which this is accomplished is beyond the scope of this essay, but a good explanation of this mode, as well as a more extensive discussion of the other modes can be found in (1). A comprehensive overview of AFM may be found in (2).

Applications in pathobiology. Reference (1) provides a bibliography of publications on the use of AFM in the study of cancer cells. Two additional articles provide detailed explanations of how to optimize AFM as well as interesting data on cancer cells (2,3). One of the major strengths of AFM is its ability to be used on living cells as can be seen, for example, in (4,5). It may be especially useful in understanding metastasis from a physical point of view. AFM has also been used successfully in the study of bacterial interaction, as reviewed in (6,7) and has provided insights into embryogenesis, as well as other systems involving cellular coordination. A volume edited by Braga and Ricci (8) discusses both principles of AFM and many biological applications as the field existed prior to 2011.

Summary and Overview. AFM is a tool for the detection of morphological and mechanical properties at nanometer levels of resolution. A basic tenet of biophysical pathology is that, regardless of underlying biochemical pathways, cells ultimately interact with their environment via physical forces. Thus these are important parameters to study in the investigation of cancer as well as other pathological processes. Other physics based probes such as MALDI imaging and Raman imaging provide information about molecular changes and molecular interactions. Surface Plasmon Resonance (SPR) studies can provide information as to cellular viscosity at regions below and in proximity to the cell membrane. MALDI has been the subject of MoM#1. Raman Imaging and SPR will be covered in subsequent MoMs. Moreover, in concert with high resolution optical methods such as confocal microscopy-based techniques, AFM should allow for the study of these parameters in subcellular organelles. Finally, it is important to note that AFM has inspired a variety of other scanning probe techniques, thus opening up many new avenues of investigation. We have focused on imaging the topography of surfaces based upon force interactions leading to information about mechanical properties of the specimen. By modifying the AFM tip it is possible to measure other properties such as electromagnetic properties and chemical potentials, and also to perform spectroscopic analysis. Some examples are chemical force, magnetic force, scanning capacitance, and, electrostatic force microscopy. While over 20,000 AFM-related papers have been published, it is only recently that investigators have turned their attention to biological systems. AFM may be especially useful in characterizing the biophysical aspects that underlie cell interaction in embryogenesis, cancer, microbiology, and stem cell biology, as well as other biologic systems of interest from the point of view of biomedicine.

Further Reading:

1. Sokolov, I. Atomic Force Microscopy in Cancer Research. In Cancer Nanotechnology, Nalwa,S. and Webster,T., editors, pp. 1-17, American Scientific Publishers, Inc. 2007.

2. Krisenko, M.O.,Cartagena, A., Raman, A. and Geahlen, R. Nanomechanical property maps of breast cancer cells as determined by multiharmonic atomic force microsocopy reveals Syk-dependent changes in microtubule stability mediated by MAP1B. Biochemistry 54: 60-68, 2015.

3. Dong, C., Hu, X., and Dinu, C.Z. Current status and perspectives in atomic force microscopy-based identification of cellular transformation. Int. J. Nanotechnology 11: 2107-218, 2016.

4. Shi, X et al. Living cell study at the single molecule and single cell levels by atomic force microscopy. Nanomedicine 7: 1625-1637, 2012.

5. Cartagena, A. and Raman, A. Local Viscoelastic properties of live cells investigated using dynamic and quasi-static atomic force microscopy methods. Biophysical journal 106: 1033-1043, 2014.

6. Liu, S. & Wang,Y. Application of AFM in microbiology. Scanning, 32: 61–73, 2010.

7. Dufrene, Y. Atomic force microscopy in microbiology: New structural and functional insights into the microbial cell surface. mBio, 5 (4) e01363-14, 2014. http://mbio.asm.org/content/5/4/e01363-14.full

8. Braga, P.C. and Ricci, D., editors. Atomic Force Microscopy in Biomedical Research: Methods and Protocols, Humana Press, Springer-Verlag, 2011.