MICROSCOPES AREN'T JUST FOR MICROSCOPISTS, ANYMORE!

Stephen W. Carmichael,[1] Mayo Clinic

Historically, microscopes have been used to gather morphologic data. We have called people who use these instruments microscopists, and it is implied that microscopists are morphologists. As was pointed out in the April/May issue of this newsletter, useful information about a specimen is also gained from temporal analysis. Further, it has been appreciated that the new family of scanning probe microscopes can be used to gather additional types of information so that these instruments have the potential to be useful beyond the dreams of a conventional microscopist. As we will discuss in this article, the future is here for one such application.

The atomic force microscope (AFM) takes advantage of the leverage afforded by the deflection of a laser beam bounced off a cantilevered stylus that is scanned over the surface of a specimen. As the stylus is deflected by a small change in the topography of the surface, the light beam begins to be deflected away from a photodetector. Piezoelectric devices in the arm suspending the cantilever correct the position of the stylus so that the light beam remains on the photodetector. A computer can then use the amount of "correction" utilized to reconstruct a map of the scanned surface with incredible resolution.

But what of the forces of attraction or repulsion between the stylus and the surface? Can these forces be measured? Could this be useful information? Can this be utilized to quantitate binding forces between biologic molecules? No doubt these questions have been asked by several "microscopists" using the AFM, but Ernst-Ludwig Florin, Vincent Moy, and Hermann Gaub appear to be the first to directly measure the forces binding two protein molecules.[2] They coated a conventional silicon nitride stylus with bovine serum albumin, attached biotin, and covered the biotin with avidin. The functionalized tip was then advanced to an agarose bead that had been covered with biotin. As the tip made contact with the bead, the cantilever was deflected. The stylus was then retracted from the bead and a deflection in the opposite direction (this was caused by the attraction between the biotin and avidin) was detected until the bonds between the molecules were disrupted and the cantilever sprang back to the neutral position. This deflection could be directly converted to force because the spring constant of the cantilever was known. Florin *et al.* presented convincing evidence that the force required to separate a single biotin molecule from avidin is 160 ± 20 piconewtons (pico = 10^{-12}). This is an impressive feat that apparently constitutes a novel application of the AFM.

Florin *et al.* go on to suggest that with modifications this technique may be used to directly measure ligand-receptor forces on the surface of a living cell. This would be remarkable, indeed! There are clearly some major problems to be overcome before this would be possible. A comparison of binding constants suggests that the forces involved in drug-receptor and antigen-antibody interactions are about three orders of magnitude lower than the biotin-avidin binding measured in this study. Difficulties created by the "springy" surface of a cell and noise imposed by thermal fluctuations (Brownian motion) are not trivial. Another method that may better measure such small forces in a living system may come from a related application of "optical-force microscopy." As demonstrated by Lucien Ghislain and Watt Webb,[3] the spring constant of an optical-force transducer is comparable to the mechanical cantilever of the AFM. It is probably just a matter of time before "microscopists" are clever enough to exploit this feature to directly measure forces between molecules.

It is evident that microscopes are moving out of their traditional roles of depicting morphology. The ability to directly measure biologic forces has been added to the armamentarium of the modern microscopist. ■

1 The author gratefully acknowledges the help of Vincent Moy, Technical University of Munich, and David Clapham, Mayo Clinic, and the members of his laboratory.

2 Florin, E.-L., V.T. Moy, and H.E. Gaub, Adhesion forces between individual ligand-receptor pairs, *Science* 264:415-417, 1994.

3. Ghislain, L.P., and W.W. Webb, Scanning-force microscope based on an optical trap, *Optics Letters* 18:1678-1680, 1993

My Microscope, Snoopy

I have a friend able to see
Objects much too small for me
And though his eyes, he lets me peak
At tiny wonders which I seek
To see as if I could go
Into that tiny world below.

He is my own light microscope
And you my friend I dearly hope
Will come to meet that friend of mine
And spend the pleasant hours of time
To see the wonders I have seen
of pond and flowers and insect wing.

Sterling Newberry
(rights reserved)

https://doi.org/10.1017/S1551929500066311 Published online by Cambridge University Press