LECTURE 6: AFM IMAGING II : ARTIFACTS AND APPLICATIONS

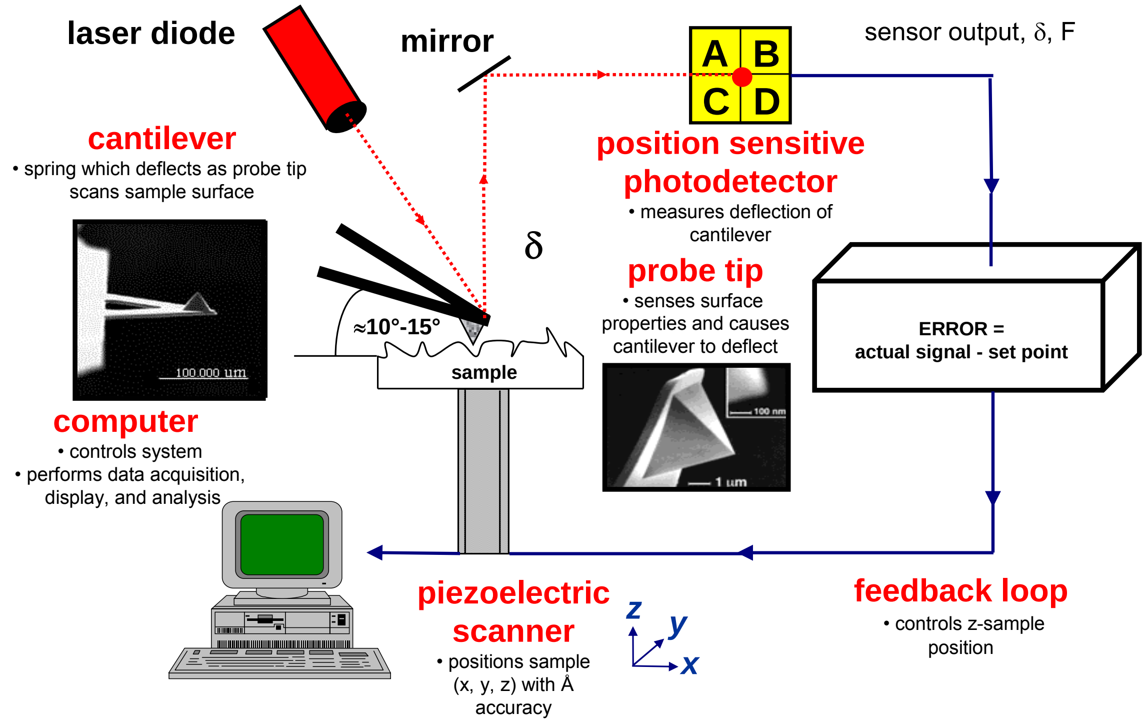
Outline :

Objectives: To review artifacts present in atomic force microscopy imaging and current state of the art uses.

Readings: Course Reader Document 12-15.

Multimedia : Listen to "Structured Water" Podcast corresponding to journal article : Higgens, et al. *Biophys. J.* **2006** 91, 2532.

ATOMIC FORCE MICROSCOPY : GENERAL COMPONENTS AND FUNCTIONS



-Basic principles

-Deflection vs. Height images

-2D Section Profiles

-3D Images

-Normal modes of operation (Contact AC/DC, Tapping AC, Noncontact AC)

-Other modes of operation (LFM, CFM, Force/Volume)

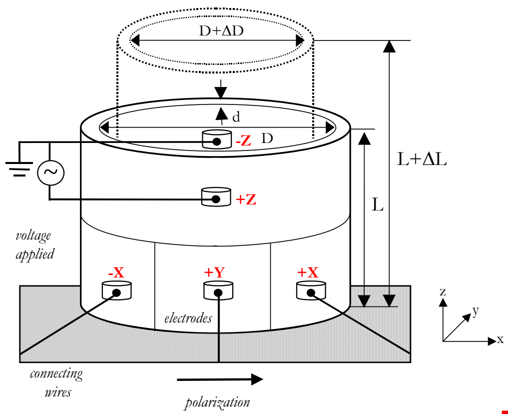
-DEMOs

Advantages : 1) Unlike electron microscopes, samples do not need to be coated or stained, minimal damage, 2) Unlike electron microscopes, samples can be imaged in fluid environments (near-physiological conditions), 3) Unlike STM samples do not need to be conductive, 4) Sub-nm resolutions have been achieved on biological samples (detailed information on the molecular conformation, spatial arrangement, structural dimensions, rate dependent processes, etc.)

ATOMIC FORCE MICROSCOPY IMAGING : FACTORS AFFECTING RESOLUTION

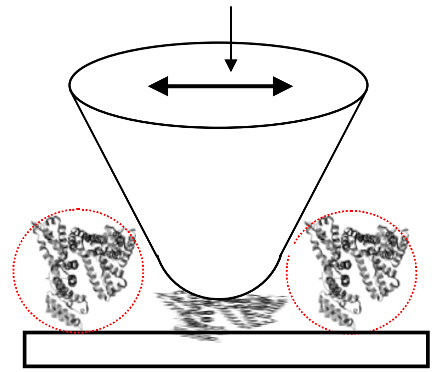
PIEZO AMPLIFIER, SENSOR AND CONTROL ELECTRONICS, MECHANICAL PARAMETERS

Physik Instruments, *Nanopositioning* **1998**



SPECIMEN DEFORMATION & THERMAL FLUCTUATIONS

Hoh, et al. *Biophys. J.* **1998**, *75*, 1076.

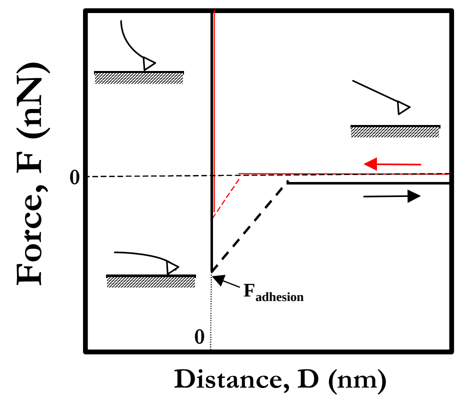


PROBE TIP SHARPNESS

Sheng, et al. *J. Microscopy* **1999**, *196*, 1.

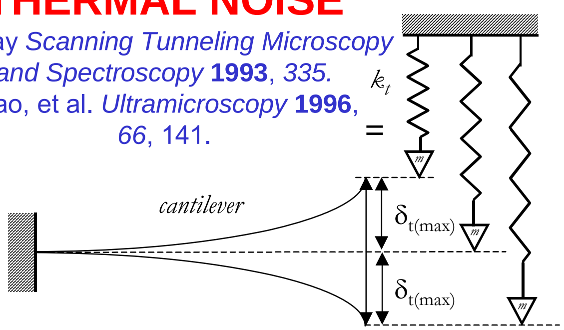
ADHESION FORCE

Yang, et al. *Ultramicroscopy* **1993**, *50*, 157



CANTILEVER THERMAL NOISE

Lindsay *Scanning Tunneling Microscopy and Spectroscopy* **1993**, *335.*
Shao, et al. *Ultramicroscopy* **1996**, *66*, 141.



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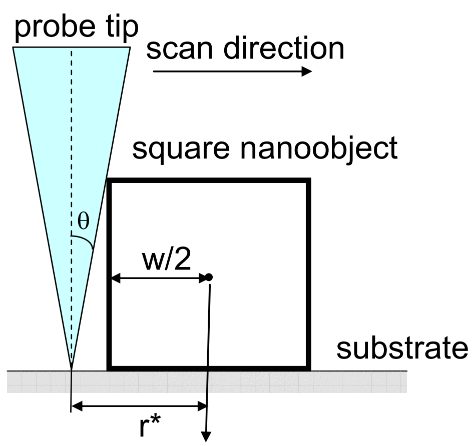
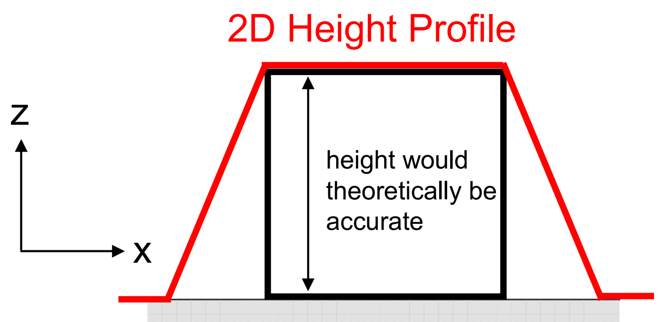
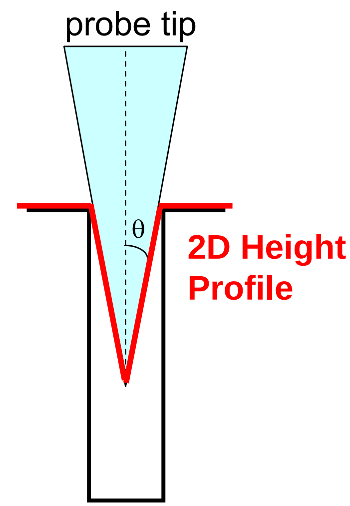
3-D model of sharp probe tip on a protein, from Lieber et al, 2000 (http://cnst.rice.edu)

ATOMIC FORCE MICROSCOPY IMAGING : TIP DECONVOLUTION

-Imaging very sharp vertical surfaces is influenced by the sharpness of the tip. Only a tip with sufficient sharpness can properly image a given z-gradient. Some gradients will be steeper or sharper than any tip can be expected to image without artifact. False images are generated that reflect the self-image of the tip surface, rather than the object surface. Mathematical methods of tip deconvolution can be employed for image restoration. The effectiveness of these methods will depend on the specific characteristics of the sample and the probe tip.







-Deep feature
-Depth underestimated
-Need high aspect ratio probe tip.

$$\tan(\theta) = \frac{x}{w} \rightarrow x = w\tan(\theta)$$

$$r^* = \frac{w}{2} + w\tan(\theta)$$

$$\frac{r^*}{w} = \frac{1}{2} + \tan(\theta)$$

-tip broadening-width is overestimated → mention pset

AFM IMAGING OF BIOLOGICAL MACROMOLECULES: DNA

Tapping Mode image of nucleosomal DNA.
Courtesy of Yuri Lyubchenko. Used with permission.

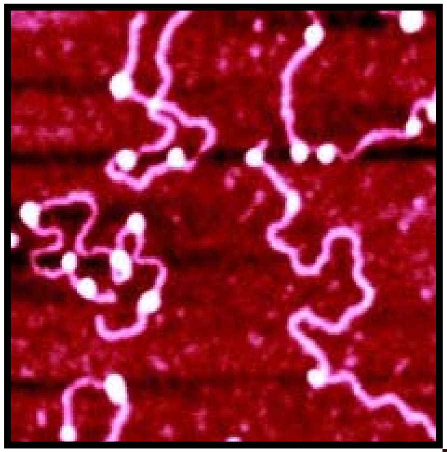
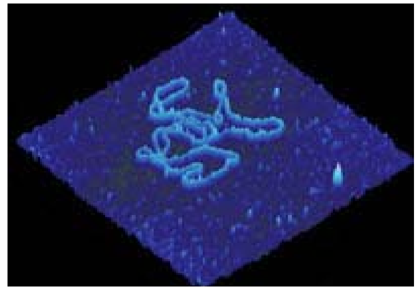
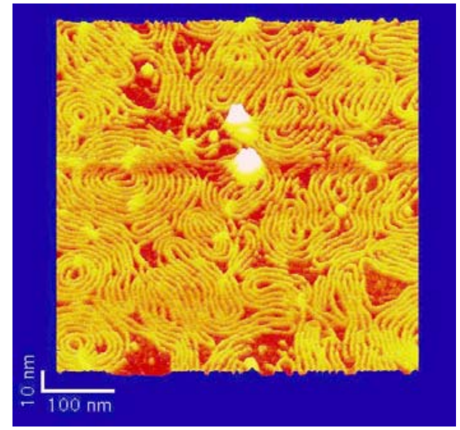


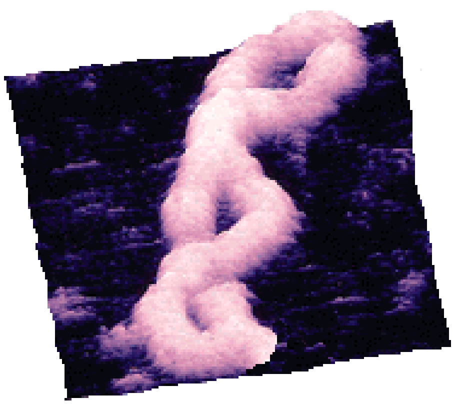
Image of PtyrTlac supercoiled DNA. 750 nm scan courtesy C. Tolksdorf, Digital Instruments/Veeco, Santa Barbara, USA, and R. Schneider and G. Muskhelishvili, Istitut für Genetik und Mikrobiologie, Germany.



Courtesy of Veeco Instruments and G. Muskhelishvili. Used with permission.

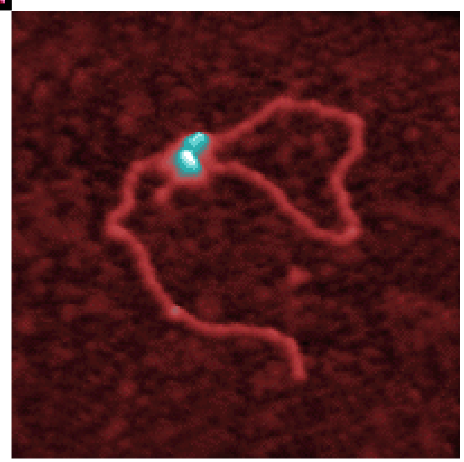


Courtesy of Zhifeng Shao. Used with permission.
http://people.virginia.edu/~zs9q/zsfig/DNA.html



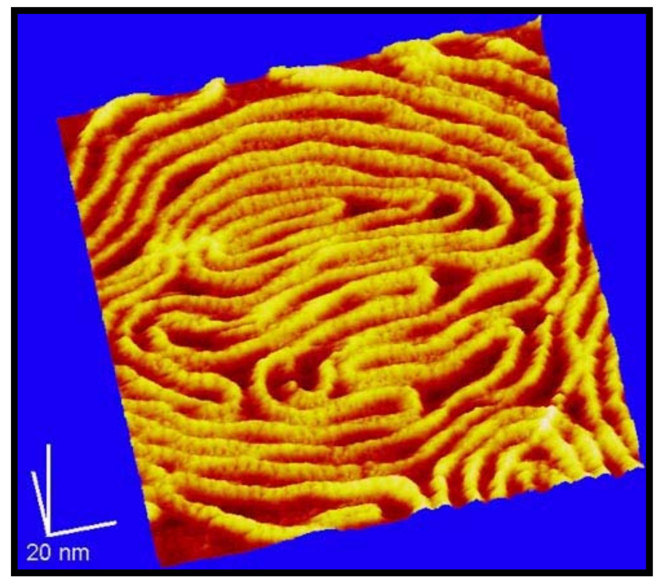
The high resolution of the SPM is able to discern very subtle features such as these two linear dsDNA molecules overlapping each other. 155nm scan.

Courtesy of W. Blaine Stine. Used with permission.



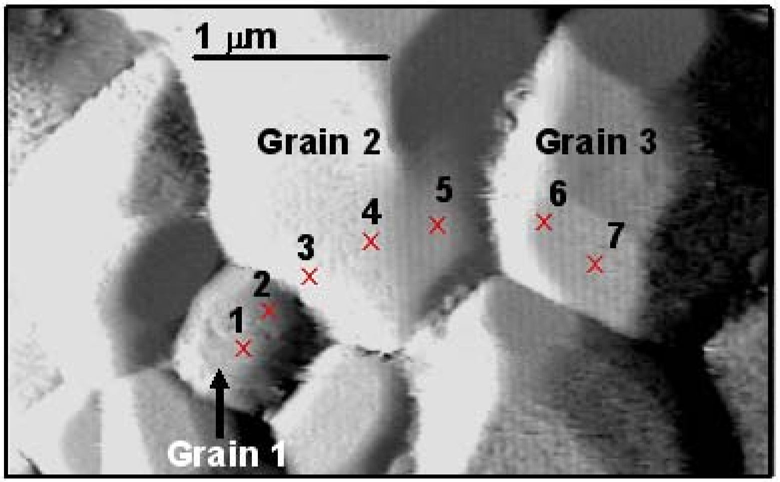
AFM image of short DNA fragment with RNA polymerase molecule bound to transcription recognition site. 238nm scan size. Courtesy of Bustamante Lab, Chemistry Department, University of Oregon, Eugene OR

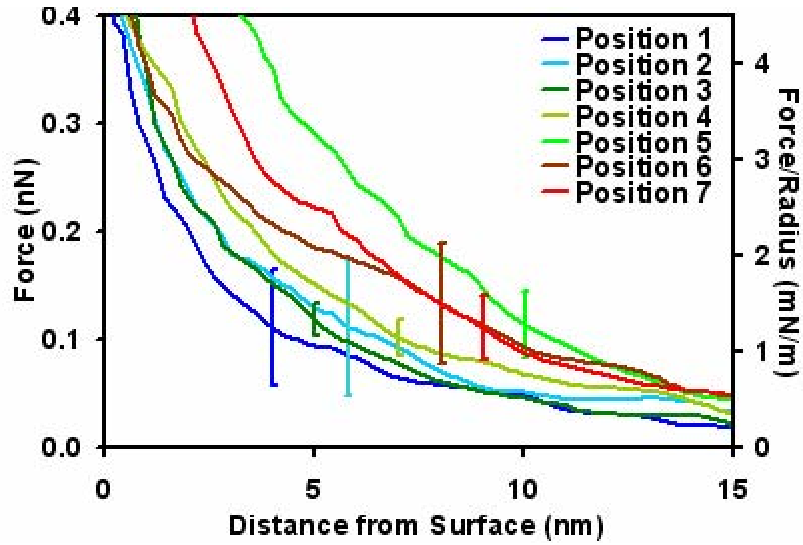
Courtesy of Prof. Carlos Bustamante. Used with permission.



Courtesy of Zhifeng Shao. Used with permission.

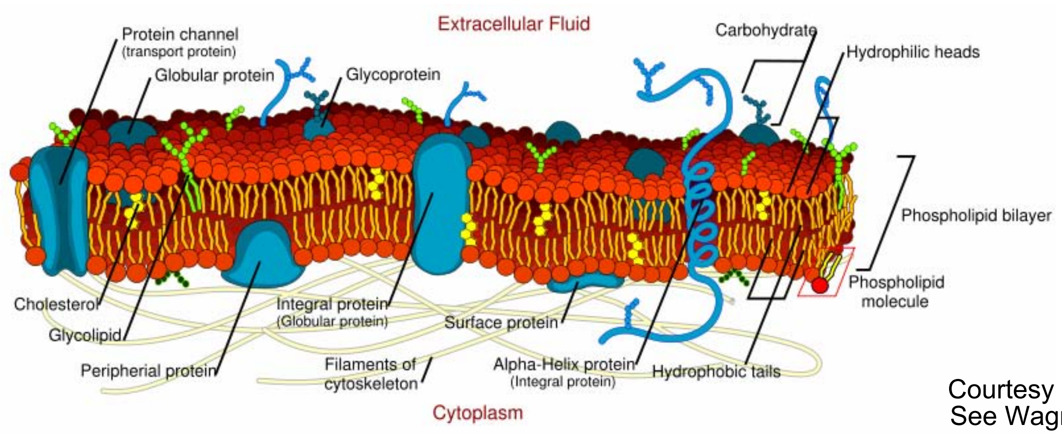
HRFS COMBINED WITH AFM : SPATIALLY SPECIFIC SURFACES INTERACTION INFORMATION



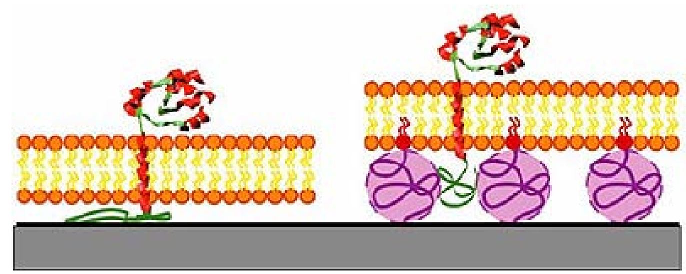


(Vandiver, et al. *Biomaterials* 26 (**2005**) 271–283).

SUPPORTED LIPID BILAYERS

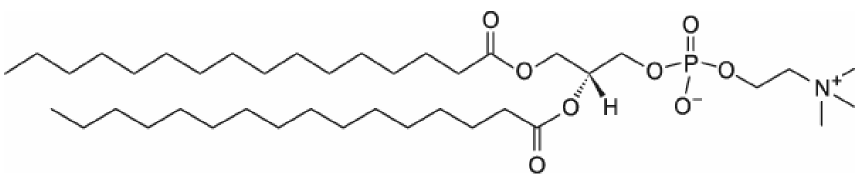


http://en.wikipedia.org/wiki/Image:Cell_membrane_detailed_diagram.svg

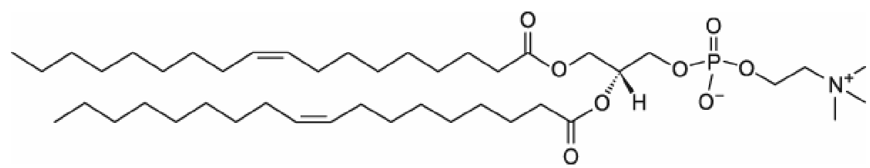


http://faculty.virginia.edu/tamm/pages/project_support.html

See Wagner, M., and L. K. Tamm. *Biophysical Journal* 79 (2000): 1400-1414.



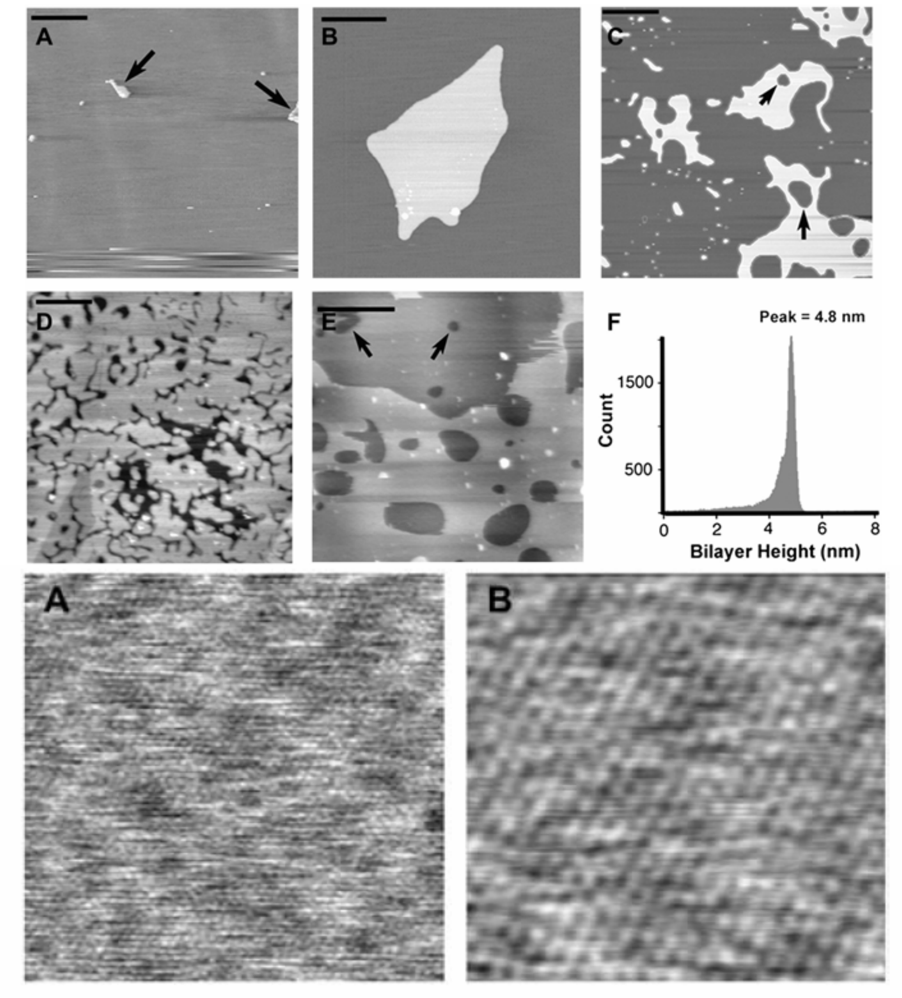
DPPC



DOPC

Higgens, et al. Biophys. J. 2006 91, 2532.

NANOMECHANICS OF SUPPORTED LIPID BILAYERS



Higgens, et al. Biophys. J. 2006 91, 2532.