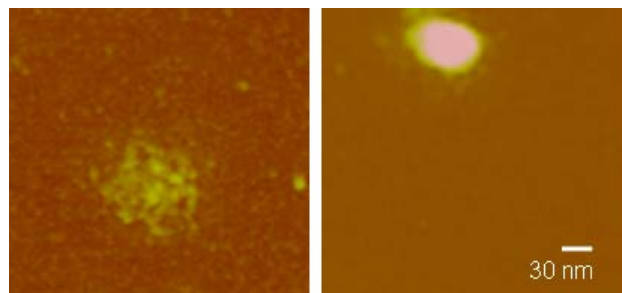


## Nanomanipulation Core Technology

The goal of this core technology platform is the application and development of nanomanipulation and nanocharacterization techniques for assembly of polymer micro- and nanofluidic devices. In particular we employ the atomic force microscope (AFM) to perform nanoscale imaging in air or in a fluid environment, to perform dip-pen-nanolithography of biomolecules on substrates and to characterize surface elasticity by AFM force measurements. We have previously demonstrated how AFM and other microscopic techniques can be used to detect and analyze the presence of magnetic nanoparticles [1], to analyze the cell surface topology of mechanically manipulated cells [2] or study the regulation of collagen fibrillogenesis at the molecular level [3, 4]. Currently, we are employing the AFM to characterize DNA conjugated polymer nanoparticles and cell-surface topology in a fluid environment and to test surface morphological and physical properties of polymer thin films and/or patterned surfaces.

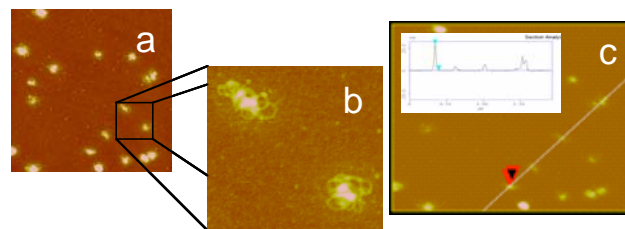
### AFM imaging of PEI-DNA particles

One of the testbeds in NSEC employs development of mammalian cell transfection protocols using DNA conjugated to polymer nanoparticles (PEI). This conjugation is achieved either by bulk-mixing DNA and PEI or through microfluidic channels. We characterized the conjugation of DNA with PEI nanoparticles using AFM imaging in ambient air or in a fluid environment (Fig. 1).



**Figure 1.** AFM images of (a) DNA alone and (b) PEI alone, imaged on mica in HEPES-Mg buffer using AFM in tapping mode.

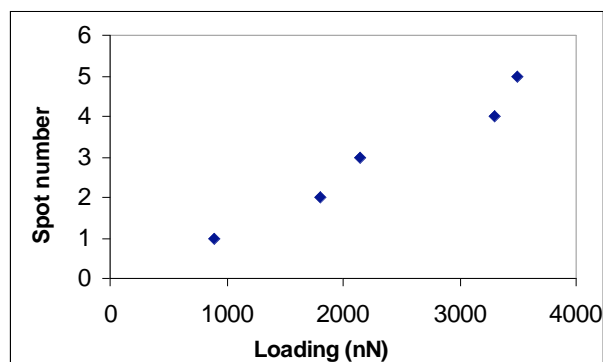
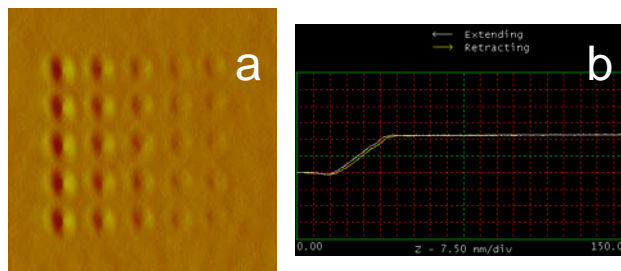
Next, PEI-DNA complexes obtained by either microfluidics or bulk mixing were imaged with AFM and the data analyzed to assess particle size and morphology and the difference between the particle size distributions (Fig. 2). Use of a  $Mg^{2+}$  supplemented buffer enabled us to circumvent problems of insufficient attachment of DNA to the mica substrate. The data collected so far suggests that the sample prepared using microfluidics samples have a more uniform size distribution than the traditionally bulk-mixed samples.



**Figure 2.** PEI-DNA complexes prepared using microfluidics were immobilized on a mica surface and imaged in a HEPES-Mg buffer environment using AFM. High resolution AFM imaging (a, b) shows conjugation of DNA to PEI and AFM section analysis (c) enables determination of particle height at nanoscale resolution.

### Nanoindentation

Thin films (of thickness 20 to 40 nm) of polystyrene spread over a silicon substrate were subjected to arrays of indentations to assess the superficial elastic vs. plastic properties of the polymer. To achieve nanoindentation on the polystyrene films, silicon cantilevers were lowered under various loading forces on the sample surface, then retracted while the force was controlled. Force displacement and deflection of the cantilever were recorded continuously.

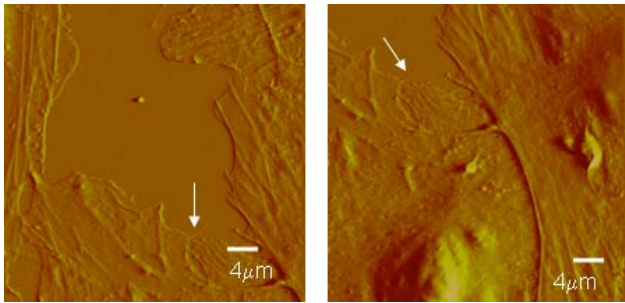


**Figure 3.** (a) Array of indentations under decreasing loading forces (left to right) with depths ranging between 0.3 nm – 1 nm. (b) Force curve analysis enables determination of surface elastic properties when the loading force for each spot in (a) is known as in (c).

As shown in Figure 3, force measurements allowed us to assess differences between the resistance forces when extending vs. retracting the AFM probe tip. By comparing these with the sub-nanometer depth of the indentation marks and the loading force applied, superficial elastic properties were assessed. Similar experiments at 37°C and 45°C have been designed to assess the dependence of these properties on temperature.

### Cell surface imaging

To analyze cell surface morphology at the nanoscale level, 3T3 fibroblast cells were cultured in 50 mm dishes and treated with 50µg/ml of ascorbate to assess the effect of collagen secretion outside the cell. Cells not treated with ascorbate were used as a control. Cells were fixed using 4% glutaraldehyde for AFM imaging in PBS. As shown in Figure 4, nanoscale surface topographical features and filamentous structures near the cell edges have been resolved using AFM imaging.



**Figure 4:** AFM images of cultured 3T3 cells showing nanoscale cytoskeletal organization and secretion of filamentous structures from the cell edges (arrows).

Further work in this direction involves investigation at the nanoscale (by AFM) along with simultaneous fluorescence imaging of cells after subjecting them to transfection methods like PEI-DNA conjugates, Membrane Sandwiched Electroporation (MSE) or liposome-based gene delivery.

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