

## Single-Biomolecule Resolution Imaging with an Optical Microscope

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A Fluorescence Apertureless Near-field Scanning Optical Microscope (FANSOM) has been developed with FWHM optical resolution below 10 nm when imaging at ~600 nm wavelengths [1]. The apparatus combines an epi fluorescence optical microscope and an atomic force microscope (AFM) to obtain single-molecule sensitivity and optical resolution limited by the sharpness of the AFM probe. The AFM probe is used to stimulate or reduce the detected fluorescence emission rate depending on the AFM tip material and the polarization of the excitation light. The probe-sample interaction is described by near-field dipole-dipole physics, resulting in a stimulated emission rate that varies by  $r^6$ . When tapping the probe over the substrate being imaged, the near-field component is sharply modulated at that tapping frequency, thereby enabling separation from the far-field background during post-processing. Images of fluorescent single-molecules taken in a physiological environment will be presented.

We are also developing probe and substrate technologies to enable FANSOM to image and time-resolve the dynamics of biomolecular interactions. The tools developed include generalized techniques for the growth and attachment of nanotubes for use in AFM imaging. With our nanotube tips we have generated 0.5 nm resolution AFM images, potentially enabling optical imaging of single-molecules with resolutions approaching 1 nm [2]. In addition these nanotube probes can be uniquely functionalized at their tips, serving as the foundation of an effort to develop single-molecule sensors. Coating these probes with Teflon has enabled fabrication molecular-scale electrical probes [3]. Silane-chemistry dip-pen nanolithography techniques have been developed for patterning glass coverslips with functional proteins, peptides, aptamers, etc. [4,5]. By combining FANSOM with these techniques we anticipate patterning functional biomolecules onto glass coverslips and then individually characterizing highly specific molecular interactions at in-vivo-like molecular concentrations. In addition, these tools will prove relevant for characterizing cellular contents and expression with single-molecule discrimination using nanoarrays and molecular circuits. Finally, techniques are being developed for patterning phospholipid bilayers for use as model membrane systems. Together, these tools should prove particularly well suited for probing bio-interface problems such as viral insertion, transmembrane protein triggering and lipid raft formation and function.

### References

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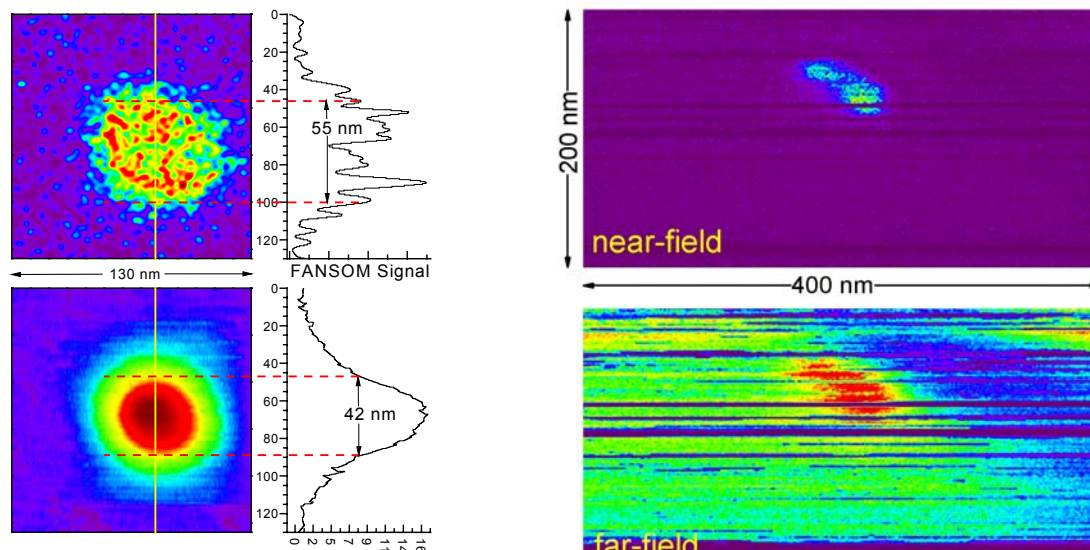


Fig 1. On the right is imaged a 17 nm diameter latex bead. In the top right the bead was imaged through fluorescence reduction using metallic tip. The simultaneously acquired AFM image is shown in the lower right. On the left are images of a 20 nm x 5 nm diameter CdSe nanorod showing the separation of near-field and far-field components via demodulation. These FANSOM images were taken through fluorescence enhancement using a silicon probe.

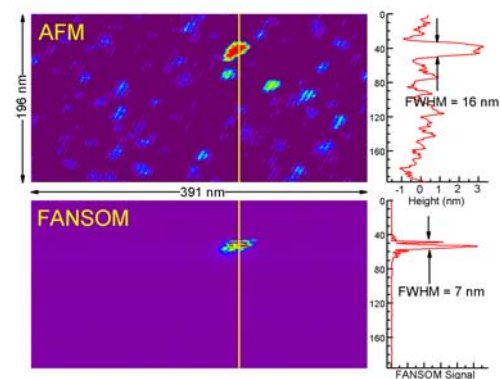


Fig. 2. AFM and FANSOM images of a 20 nm x 5 nm diameter CdSe nanorod are compared. A commercial silicon FESP probe was used for these images. The FANSOM image used a 40 nm wide filter centered at 620 nm at the detector.

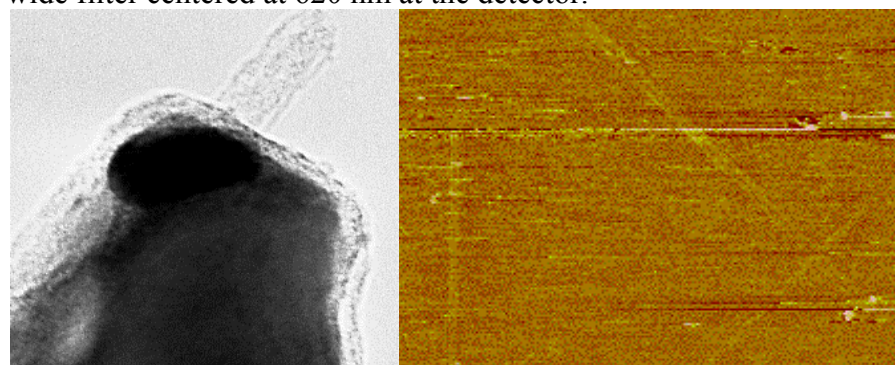


Fig. 3. On the right is a TEM image of a single-wall nanotube attached to the tip of a silicon AFM probe. On the right is a 4 x 3 μm lateral force AFM image of biotin patterned on a glass substrate. The two diagonal lines are 70 nm wide and centered 200 nm apart as are the two left lines.