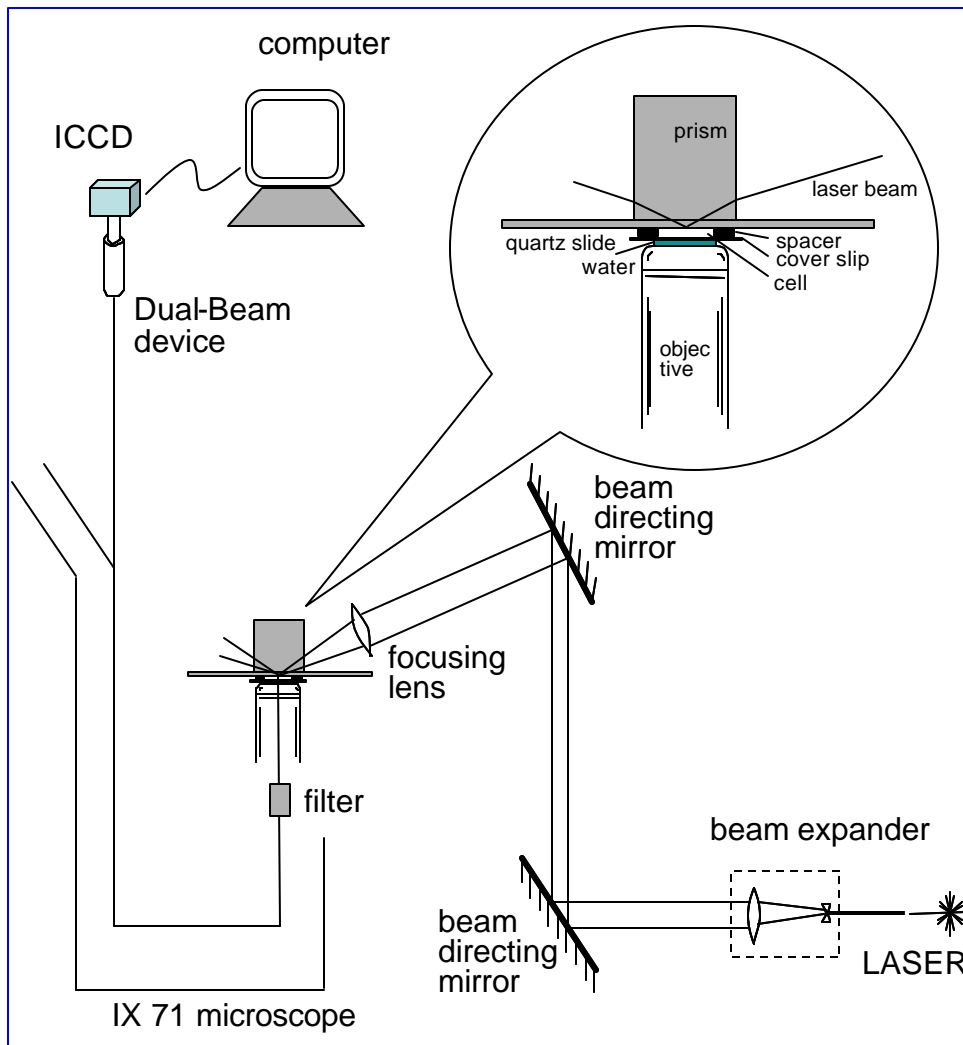


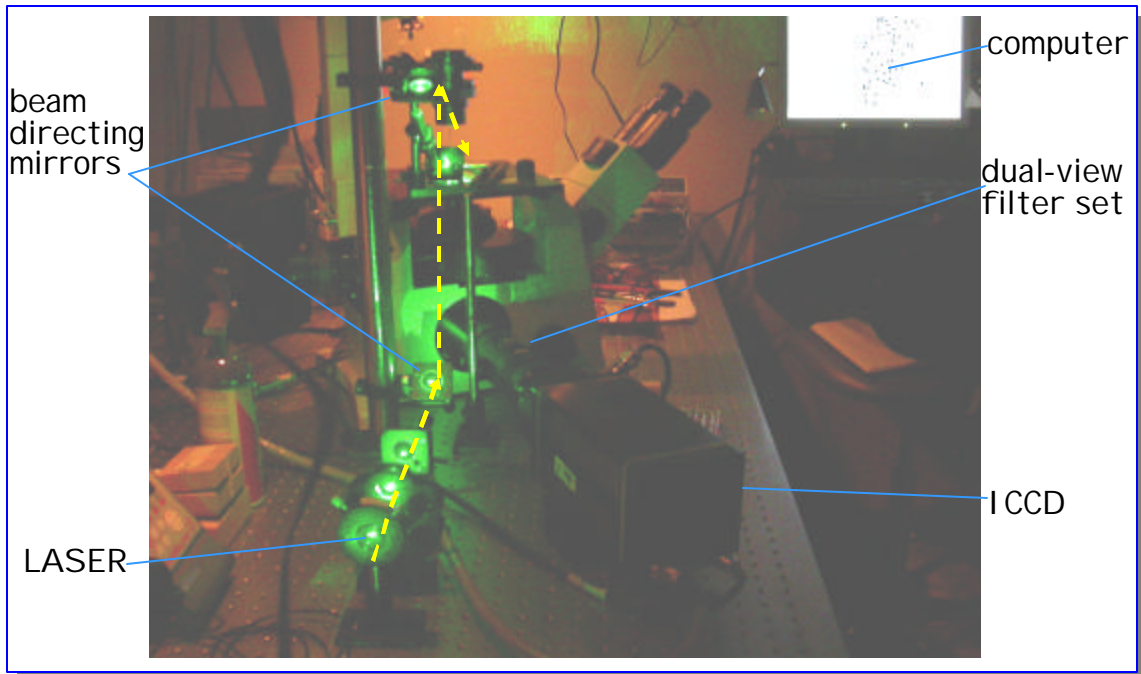
Instrumentation:

Evanescent Field Fluorescent Microscope (EFFM)

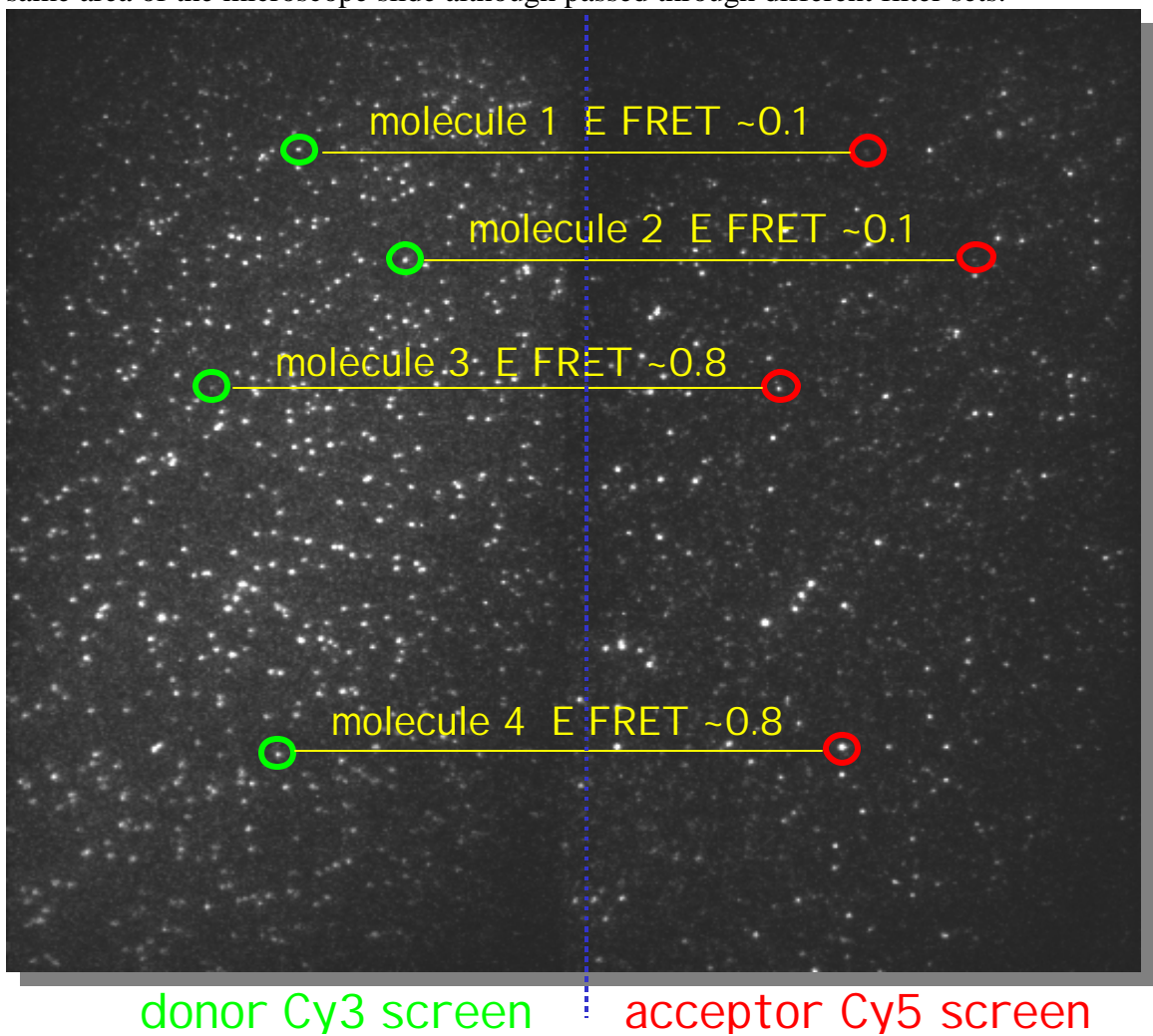
We have assembled an EFFM for prism-based wide-field fluorescence microscopy.

Schematic of EFFM





Here is an example of how we calculating EFRET distributions for populations of single nucleosomes. The left (100 microns x 200 microns) and right sides of the image are the same area of the microscope slide although passed through different filter sets.



We have used this wide field fluorescence microscope for following the dynamics of individual nucleosomes:

Fluorophore positions on naked DNA and nucleosomes

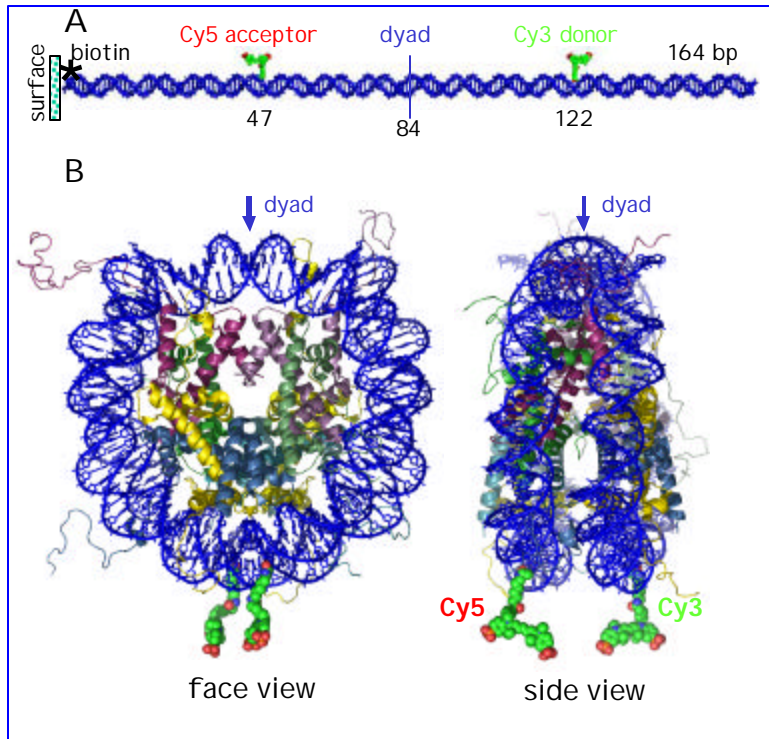
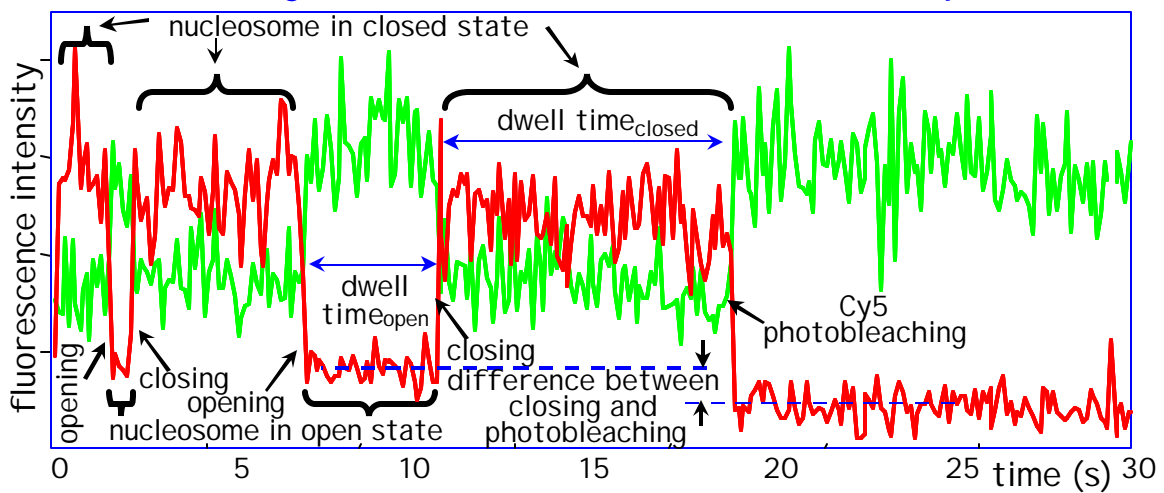


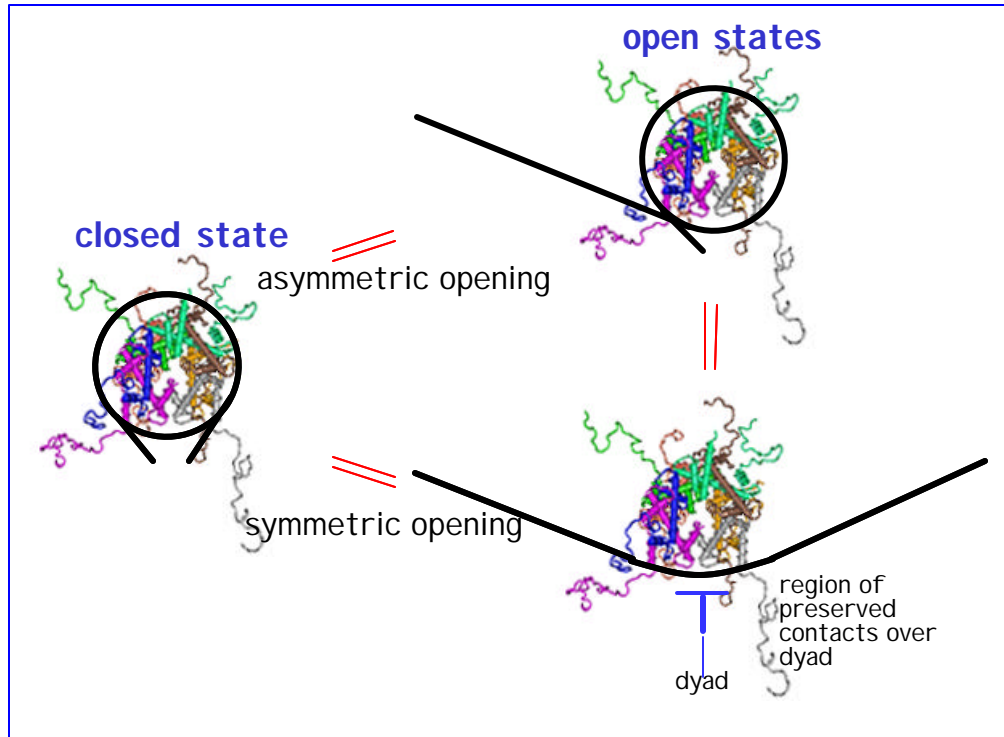
figure courtesy of Dr. Joel Harp

Time trajectories of individual fluorophores



Based on our work we found long-range, fast reversible conformational fluctuations in nucleosome particles:

Long-range, fast, reversible conformational fluctuations in nucleosome particles (nucleosome **opening**)



References:

Zheng, H., Tomschik, M., Zlatanova, J. and Leuba, S. H. Evanescent field fluorescence microscopy (EFFM) for analysis of protein/DNA interactions at the single-molecule level. *In* Protein-protein interactions, a molecular cloning manual, 2nd ed. (E. Golemis and P. Adams, Eds.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor (by invitation) Ch. 20, pp.1-16, 2005.

Tomschik, M., Zheng, H., van Holde, K., Zlatanova, J. and Leuba, S. H. Fast, long-range, reversible conformational fluctuations in nucleosomes revealed by spFRET. *Proc. Natl. Acad. Sci. USA* **102**, 3278-3283, 2005. Highlighted in *Biophotonics International* (March 2005 issue, p. 10).