NEAR-FIELD SCANNING OPTICAL MICROSCOPY:
ALTERNATIVE MODES OF USE FOR NSOM PROBES

David S. Moore-Nichols and Robert C. Dunn

1. INTRODUCTION

Near-field scanning optical microscopy (NSOM) is a scanning probe technique with a potential for revealing novel insights into the natural world at the sub-microscopic level. The technique circumvents the classical diffraction limit that constrains the spatial resolution of conventional light microscopy, unlocking new opportunities for probing sample optical properties at the mesoscopic dimension.

NSOM relies on the formation of a sub-wavelength sized light source that is scanned in close proximity to a sample surface. The resulting spatial resolution in such a configuration is limited only by the size of the light source and its position near the sample. (Betzig, Trautman et al. 1991; Pohl 1991) Resolution is not limited by the wavelength of illumination as it is in microscopy techniques utilizing conventional optical lenses. For example, Figure 1 shows a NSOM fluorescence image of single molecules in a lipid film. Each bright spot represents the fluorescence from a single fluorescent molecule in the film, illustrating the low detection limits possible with this technique. Moreover, the full-width-at-half-maximum of each feature is approximately 28 nm, demonstrating the high spatial resolution possible with NSOM. (Hollars and Dunn, unpublished data.) To further illustrate this point, the image size presented in Figure 1 is 514 nm; the same dimension as the excitation wavelength used in this measurement. Thus, NSOM is clearly capable of greatly exceeding the resolution limits of traditional methods of light microscopy.

The various methods used in forming sub-wavelength apertures and implementing NSOM techniques have been the subject of several recent reviews and will not be recounted in this chapter. (de Lange, Cambi et al. 2001; Dunn 1999; Edidin 2001; Higgins 2002; Lewis, Radko et al. 1999; Shiku and Dunn 1999) Moreover, readers are
Figure 1. NSOM image of single fluorescent molecules in a DPPC lipid film created using the Langmuir-Blodgett technique. Each bright spot represents the fluorescence from a single fluorescent molecule of dilC18 in the film, illustrating the low detection limits possible with this technique. The full-width-at-half-maximum of each feature is ~28 nm, (black arrows) demonstrating the high spatial resolution that is possible with NSOM. The image size presented in Figure 1 is 514 nm, the same as the excitation wavelength, illustrating the high resolution achieved with NSOM.

referred to an excellent chapter within this edition for a discussion of NSOM fluorescence measurements which, to date, have proven to be the most popular and informative of the NSOM contrast mechanisms. This chapter will instead provide a selective overview of some non-traditional uses of NSOM probes in scanning probe microscopy and molecular detection. Descriptions of the NSOM methods found here are not meant to reiterate the expanding spectroscopic applications of NSOM, but are rather meant to introduce NSOM variants permitting sample characterization not typically accessible by traditional scanning probe techniques. While approaches covered in this chapter continue to take advantage of the sub-wavelength light source of traditional NSOM probes, these modified techniques offer enhanced or unique contrast mechanisms that expand the information available to this form of scanning probe microscopy.