We recall that our aim is to investigate, on the molecular level at a spatially resolved mode of operation, structure-activity relations of DNA and their sensitivity to ionizing radiation. This entails in-vitro (and later in-vivo) ultra-resolved microscopy, spectroscopy and chemical sensing, with non-destructive probing.

1. Scanning Tip Optical Microscopy: We have equalled the highest ever reported spatial resolution (reported recently by Bell-ATT Labs) in both transmission and fluorescence microscopy of about 25 nm, i.e., at least 10 times better than confocal microscopy with visible light. This operation relies on our nano-fabrication of single-mode optical fiber tips, with extremely high brightness. Example of transmission and fluorescence microscopy are given in Figs. 1 to 3, including Rat-liver DNA images.

2. Nanospectroscopy: Electronic spectroscopy with subwavelength resolution is a promising tool for spatially resolved physical chemistry measurements. Essentially the same technology is used as for microscopy, with the addition of optical dispersion elements, i.e., gratings or interference filters. A sample spatially resolved spectrum is given in Fig. 4.

3. Fiber-Optic Chemical Sensors: Using a new technology we have constructed sensors that are a thousand times, or more, smaller, more sensitive, and faster than found in the most recent literature. Our combination of fiber-tip nano-fabrication and photopolymerization is a significant technological advance. It makes possible submicron, non-perturbative, intra-cell, opto-chemical sensing. Our sensitivity is on the order of a zeptomole ($10^{-21}$ moles) for hydrogen ions (pH measurement). Figures 5-7 illustrate the sensors, their intra-cell application and the resulting chemical information.

4. Exciton Supertips: To achieve the ultimately possible resolution (about one nm), with exciton microscopy, we have been working on the production of exciton tips and
supertips. For instance, at the exposed 40 nm area of an optical fiber tip we grow a nanocrystal (e.g., anthracene) and at its surface we deposit an even smaller amount of a "parasitic" crystallite or cluster (e.g., perylene). Due to energy transfer, the parasitic crystal (perylene) plays the role of the reaction center in a photosynthetic antenna, i.e., it sucks-in the electronic excitations of its environment. This "supertip" is expected to be the probe for scanning exciton microscopy. A preliminary Z-scan is shown in Figure 8.

5. Problems: The absence of a feedback mechanism (calibration of the Z-distance) is a well-known problem. We are currently adapting the lateral-force technique which has been first described this year at the Los Angeles meeting of the Society of Optical Engineers (SPIE, 1992). Its resolution of about 2-10 nm is perfect for near-field optical microscopy. We note that our higher-resolution exciton microscopy technique has its built-in feed-back (excitation quencing) with a resolution of 0.5-10 nm.

The bleaching of dyes (fluorophores) attached to a sample (e.g., DNA) in fluorescence microscopy is a well-known problem. We have moved to stabler dyes (e.g., Toto-1 from Molecular Probes) and to lower energy ("redder") excitation. This problem has been completely solved for our optical sensors (one sensor can be used tens of thousands of times), but not yet for DNA, where sample preparation (using anti-oxydants) or environmental control (nitrogen or helium) are under investigation.

6. Promises: With our new lab, new team (including analytical-chemistry, applied-physics, bio-physics and physical-chemistry students) and assembled equipment, we believe that definitive studies on DNA folding and related phenomena will start before or during the coming year, using optical microscopy and spectroscopy at a 20-40 nm resolution. Specifically, we plan to improve on the stability of the attached fluorophores and on the in-vitro immobilization of DNA (gels? reduced temperature?). We also have under construction a novel piezoelectric feedback scheme, using lateral force (see also Betzig et al., Appl. Phys. Lett. 60, 18 May 1992, p. 2484). The DNA imaging will concentrate mainly on chromatin structure and arrangement in chromosome-using samples
from Michigan and possibly from LBL. We also expect to put into operation our second stage of investigations—exciton and energy transfer microscopy and spectroscopy at 1 - 10 nm spatial resolution. We note that exciton fluorescence microscopy is orders of magnitude more efficient than ordinary fluorescence microscopy. Here the challenge is in nano-manipulation, i.e., mostly in learning existing techniques from our Applied Physics and Engineering colleagues.

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