Abstract: A novel concept for noninvasive high-resolution confocal biosensing based on simple apertureless fiber-optic confocal designs including either dual-confocal or single-fiber sensor systems is developed. The method can be employed for precise micron/submicron sensing the optical properties of various tissue layers and bulk samples.

Key words: Confocal Microscopy, Fiber Optic Biosensors, Noninvasive Optical Sensing

1. Introduction
Fiber optic biosensors is an intensively active field of modern biophotonics technologies because these sensors offer a noninvasive, highly sensitive, and accurate means of measurement and control of various physical and biomedical quantities. Recently, confocal laser microscopy also has been extensively applied because it ensures sharp, three-dimensional high-magnification imaging and sensing of thick specimens by rejection of out-of-focus information. To improve the spatial resolution of confocal systems, a fiber-optic approach is effectively applied offering a number of advantages in terms of spatial resolution, flexibility, miniaturization, immunity to external influences, scanning and multiplexing sensor potential.1-3 Here, we present a novel concept for biosensing optical properties of tissue samples using fiber-optic confocal designs with micron/submicron resolution.

2. Confocal Biosensor Principles
In the subwavelength nanometric scale range, where there has been recently a great impetus to obtain quantitative chemical information at cellular and intracellular level, the optical imaging/sensing techniques have a major drawback related to their spatial resolution which results from the fundamental “Rayleigh diffraction resolution limit” that is theoretically half the wavelength of the operating radiation. In the case of confocal microscopy, the diffraction limit effects on the out-of-focus light. A pinhole-based confocal microscope attempts to remove this light with a confocal pinhole aperture, but such an aperture would need to have a diameter close to zero to eliminate the out-of-focus contribution. An effective way to avoid the resolution limit and to work far beyond it is a fiber-optic based confocal microscope approach to be exploited which is the basic confocal principle of the suggested ultrahigh-resolution confocal sensing designs.

We have developed various high-resolution fiber-optic confocal sensing and imaging designs that include either dual-confocal or single-fiber sensor systems.
A noninvasive combined dual-confocal fiber-optic technique for multi-parameter sample sensing is illustrated in Fig. 1. This is a dual-confocal sensor system that includes two identical confocal microscopes used as independent sensor channels. The microscopes are based on a fiber-optic-type apertureless confocal arrangement that excludes either the input or output aperture, unlike the conventional pinhole-type confocal microscope. The combined dual-confocal fiber-optic design provides direct measurement in absolute units of refractive-index and thickness of optically transparent and non-transparent media.

Fig. 2. Basic optical design of an ultrahigh-resolution confocal fiber-optic biosensor/imaging system

To improve the dynamic range of the resolving laser power and to achieve a high resolution in the nanometric range, we have designed a simple apertureless reflection confocal microscope with a highly sensitive single-mode-fiber confocal output shown in Fig. 2. The fiber-optic design features specific advanced properties that can be summarized as follows: (1) the design is an effective alternative to conventional pinhole-based confocal systems and offers a number of advantages in terms of spatial resolution, scanning potential and miniaturization; (2) the design is compatible with a differential confocal pinhole microscope approach that allows to work beyond the diffraction limit in the nanometric scale; and (3) the design employs tools and detecting techniques that possess high signal-to-noise potential and provide a nanometric spatial resolution. This ultrahigh-resolution confocal approach can be used as a simple, accurate and noninvasive method for biosensing and imaging of tissue samples at cellular and intracellular levels as well as for precise testing of bulk samples such as measurement dioptic power of positive and negative intraocular lenses.

3. Results and Discussion
Due to the typically high spatial sensitivity of the fiber-optic confocal approach, the dual-confocal sensor (Fig. 1) ensures high accuracy in spatially locating the image confocal points and objects (<1 µm), and therefore simultaneously measuring the refractive index and thickness in absolute units. This system might be used for precise measurement of optical properties of the various tissue layers and determining small changes in these properties between cancer and non-cancer tissue areas. Combining the advantages of ultrahigh-resolution fiber-optic confocal microscopy (Fig. 2), we can work beyond the diffraction barrier in the subwavelength (below 200 nm) range exploiting confocal nanobiosensing and imaging of single-cell and intracellular analytes.

4. Conclusion
We have demonstrated an alternative high-resolution confocal biosensing method based on simple fiber-optic confocal designs that have applications in both cellular and bulk tissue biosensing.

References: