

## Nanosensors for Monitoring Molecular Signaling Pathways in a Single Living Cell

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Monitoring cellular signaling pathways inside single intact cells is becoming increasingly important fundamentally because cells in a population respond asynchronously to external stimuli. There is a need to further our understanding of basic cellular signaling processes associated with disease in order to obtain new information that is not available from population-averaged cellular measurements. A further advantage of single-cell assay is to understand the exact pathways by which signaling pathways move through the architecture of the cell. In addition, many cellular signaling pathways act on timescales of a few seconds and there is critical need for single-cell measurement techniques with similar time resolution. Not only is there a need to temporally resolve such measurements, there is also a need to spatially resolve them. For these reasons, progress in cellular physiology requires new measurement strategies at the nanoscale level applied to individual cells with great temporal and spatial resolution.

For this purpose, we have developed a new generation of nanosensors and nanoprobe combining bio-recognition and nanotechnology for *in vivo* monitoring of biochemical processes in a living cell [1, 2]. This technique could provide unprecedented insights into intact cell function, allowing, for the first time, studies of molecular functions in the context of the functional cell architecture in an integrated system approach. This presentation describes two areas of research related to the development of nanoprobe and nanosensors for single-cell analysis and imaging: (1) nanoprobe for surface-enhanced Raman scattering (SERS) biochemical analysis, and (2) nanosensors for *in vivo* analysis of a single cell.

The first research approach involves the development of metallic nanostructures that can produce the SERS effect for ultrasensitive biochemical analysis. The intensity of the normally weak Raman scattering process is increased by factors as large as  $10^6$ - $10^{11}$  for compounds adsorbed onto a SERS substrate, allowing for ultra trace-level detection. These substrates can generally be fabricated as silver-coated nanoprobe (300-nm diameter) that are capable of enhancing the Raman signal of adsorbed compounds (Figure 1). The development of a SERS gene probe technology based on the solid nanostructures is described. Sensitive and selective detection of HIV DNA and BRCA1 breast cancer gene using the SERS technology is discussed [3].

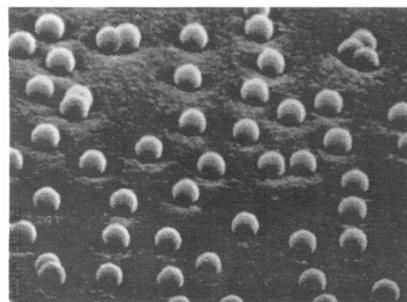


Fig. 1: Silver-Coated SERS Nanoprobe

Recent advances in nanotechnology leading to the development of optical fibers with nanoscale dimensions have opened new horizons for intracellular measurements in living cells. For example, an antibody-based nanosensor was developed to monitor benzopyrene tetrol (BPT), a DNA-adduct biomarker of human exposure to the carcinogen benzo[a]pyrene. Interrogation of single cells for the presence of BPT was carried out using antibody nanoprobe for excitation and a photometric system for fluorescence signal detection. Figure 2 shows a photograph of an