Nanobiosensors for Intracellular Analysis

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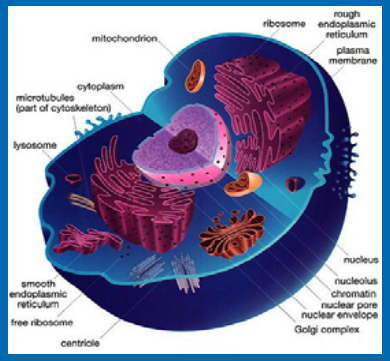
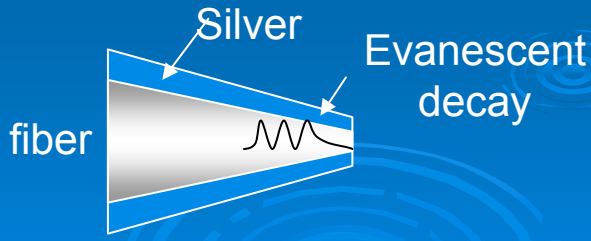
Introduction

- Many of the subcellular architectural features of the cell are on the scale of tens or hundreds of nanometers in size. Thus nanometer-sized probes can potentially be used to interrogate cellular activity in highly localized regions of the cell.

- The optical nanosensor is able to interrogate a very localized area in the immediate vicinity of the probe's tip (~ 100 nm) due to the constraints of the evanescent field excitation associated with the nanometer-scale tip, This nanosensor thus achieves an important requirement for intracellular probe design.



Schematic of animal cells
(Ref:http://www.animalport.com/img
/Animal-Cell.jpg)



Mode propagation in a tapered metal-coated optical fiber

Introduction

- Fiber-optic nanobiosensors are optical nanoprobes which have biorecognition molecules (i.e. antibodies, peptides) immobilized on their tips.
- When analyte molecules (proteins) are bound by the biorecognition molecules, changes in the fluorescence properties of light can be detected.
- Fiber optic nanosensor technology has the potential to produce a new generation of nanobiosensors able to detect specific protein targets at the single-cell levels.

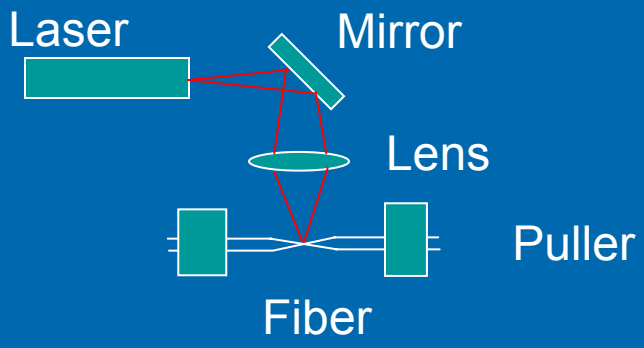
Nanoprobe for early detection of biomarkers in breast cancer

- Development of breast cancer is thought to be a multi-step process resulting from accumulation of cellular damage.
- Signal transduction pathways and transcription factors have become attractive targets of chemoprevention, given their roles in breast cell growth and their responses to steroid hormones.
- Improved understanding of how cell signaling in the breast is altered could facilitate the development of more satisfactory preventive agents and risk assessment strategies.

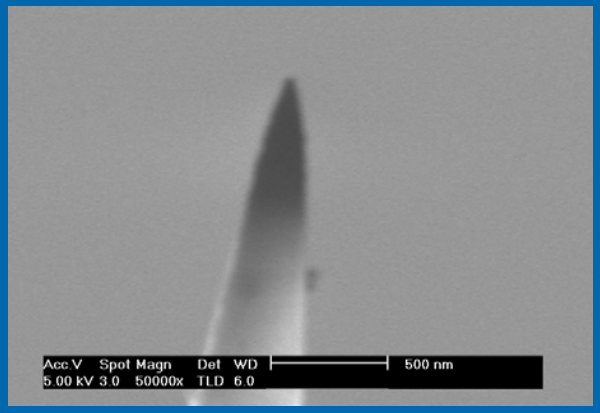
Nanosensor for caspase detection

- Nanosensor has been developed for monitoring the onset of the mitochondrial pathway of apoptosis by detecting enzymatic activities of an indicator caspase.
- Photodynamic therapy (PDT) protocols employing δ-aminolevulinic acid (5-ALA) are an established means of inducing apoptosis in MCF-7 cells.
- LEHD-AMC as a caspase-9 substrate was covalently attached to the nanosensor. Free AMC was generated by cleaving the substrate during apoptosis. By quantitatively monitoring fluorescence signals, caspase-9 activity within a single living MCF-7 cell was detected.

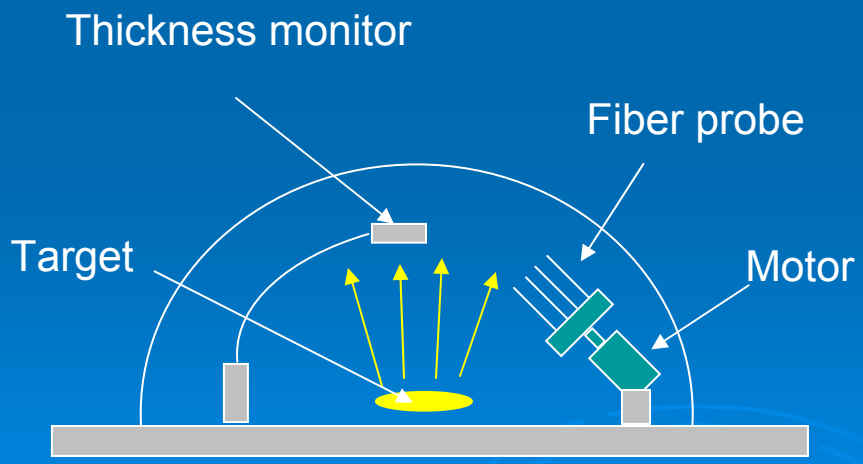
Nanoprobe fabrication



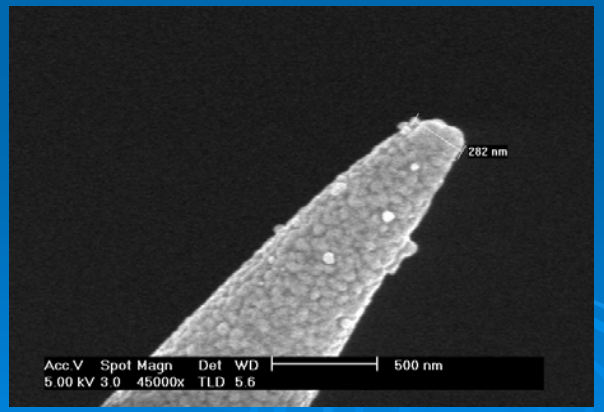
Laser-based fiber pulling



laser-pulled nanotip

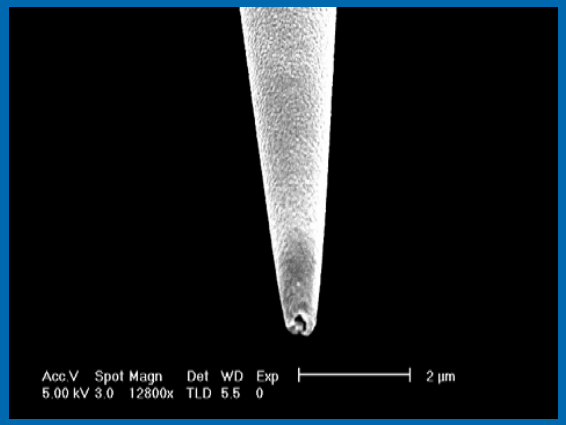


Angled evaporation



Silver coated nanotip

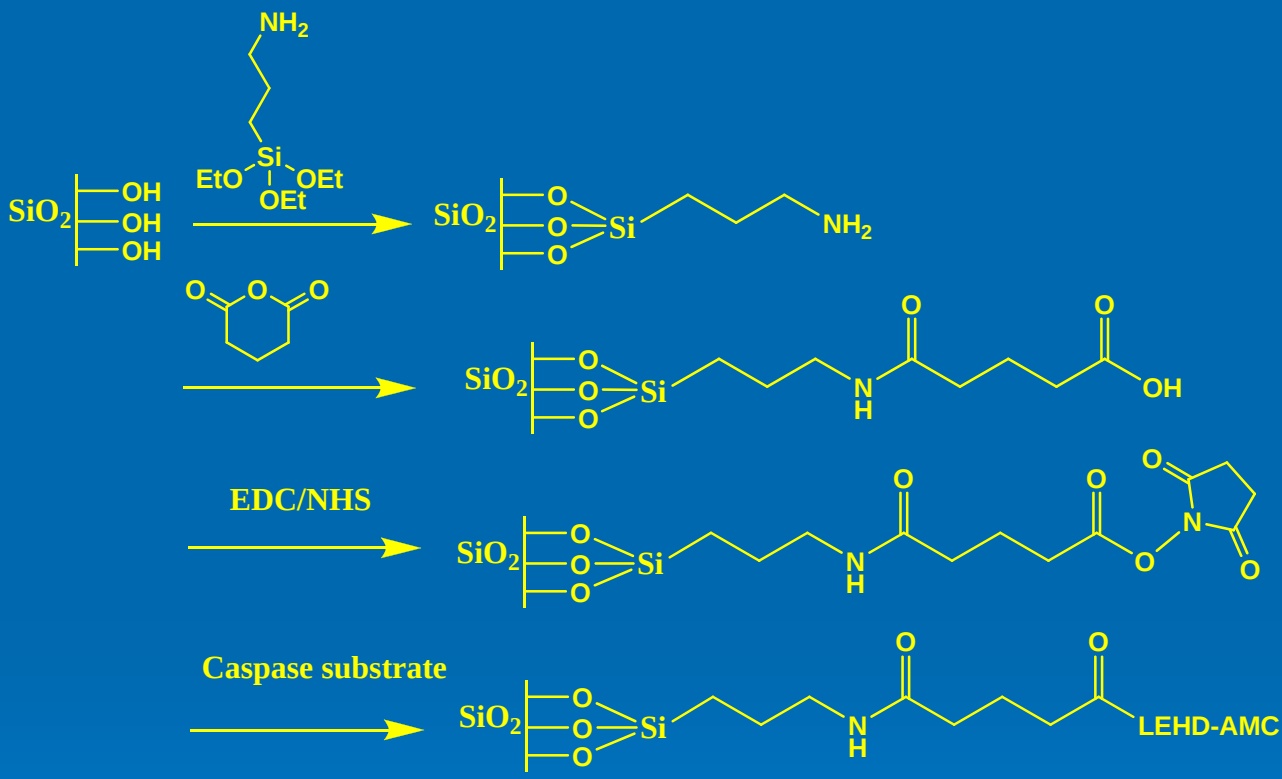
Nanoprobe fabrication



Nanoaperture fabricated by angled evaporation

Nanoaperture fabricated by focused ion beam (FIB)

Functionalization of nanoprobe



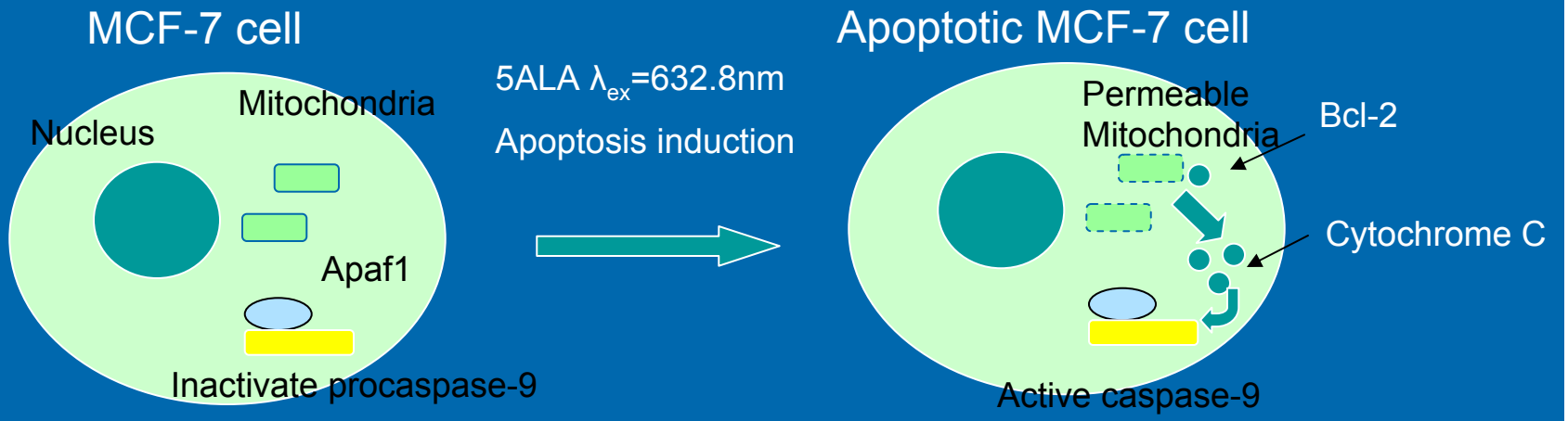
Amino group

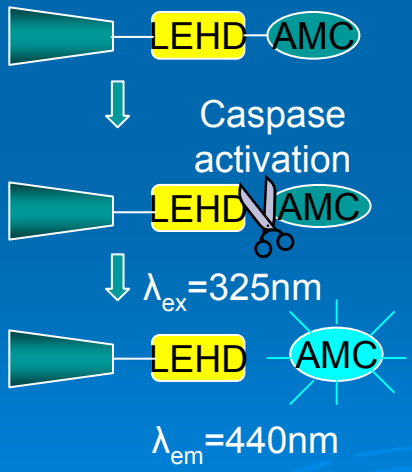
Carboxyl group

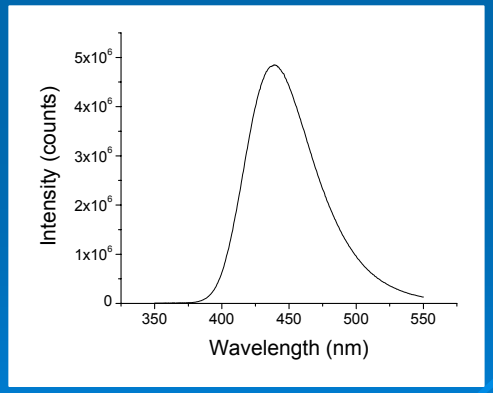
Intermediate

Caspase substrate

5-ALA induced apoptosis







Diagrammatic representation of 5-ALA induced apoptosis, involving the activation of caspase-9 followed by the cleavage of LEHD-AMC, and subsequent detection of free AMC

Nanosensor detection in single cell

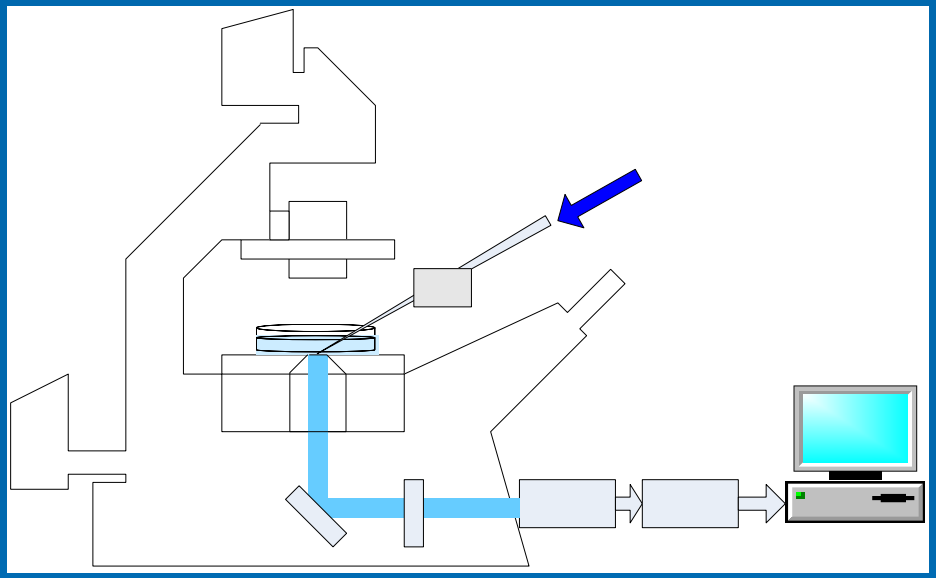


Figure 5. Schematic diagram of fluorescence measurement system

Microscope

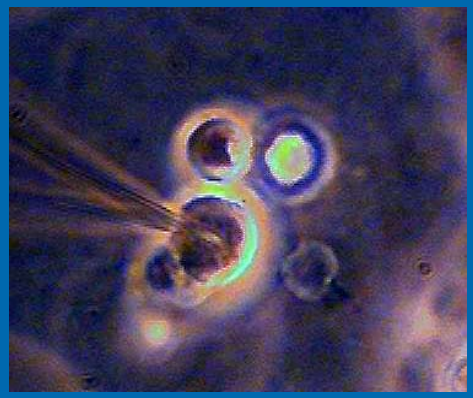
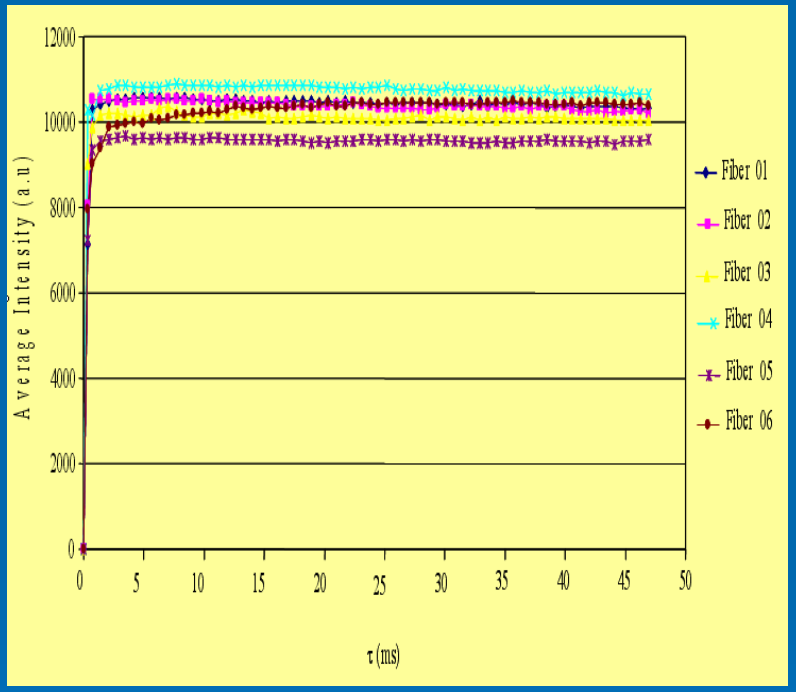


Figure 6. Phase contrast image of a nanosensor inside a living cell.

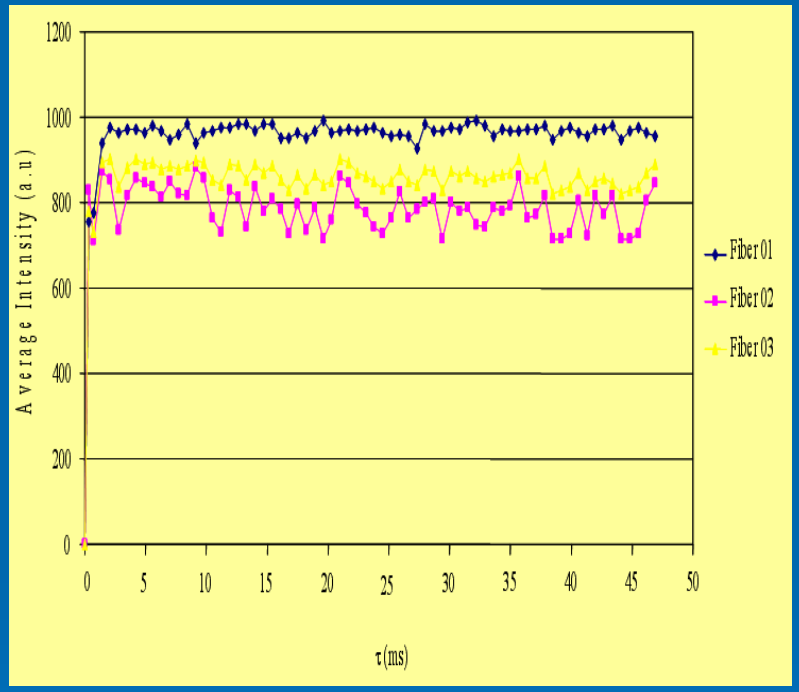
Excitation Laser

Micromanipulator

Caspase-9 activity in living cell



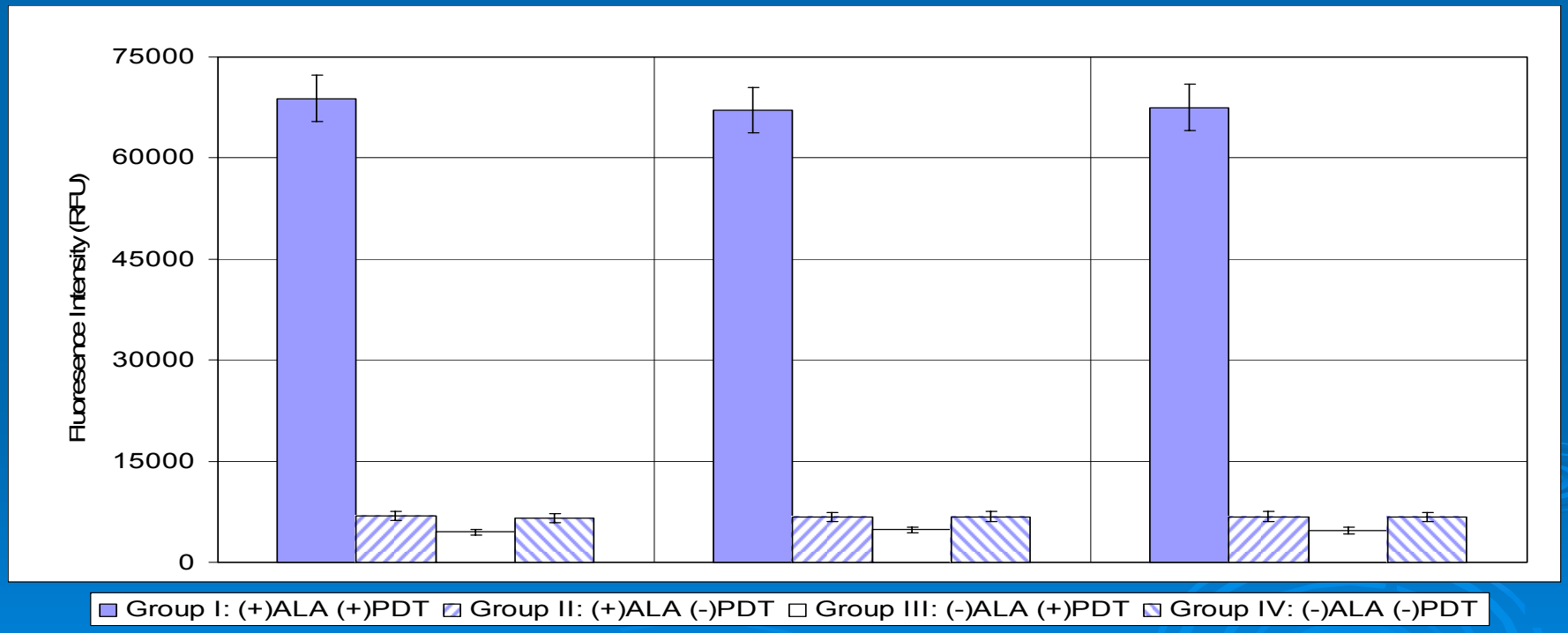
(A)



(B)

(A) The background-corrected results of intracellular detection of caspase-9 activity in experimental group of MCF-7 cells using six replicate nanosensors, one for each measurement. (B) The background-corrected results of a series of three intracellular measurements performed with a nanosensor inserted into single live MCF-7 cells of the treated control group.

In vivo measurement of Caspase-9 activity



Conclusion

- Fiber optic-based nanosensors have been utilized to study individual cells without having to disrupt their physiological makeup, which in the process can negatively interfere with cellular biochemistry.
- These nanodevices could also be used for advanced biosensing systems in order to study in situ intracellular signaling processes.
- Nanobiosensor will lead to significant advances in the diagnosis and prevention of disease at the molecular level.

Future work

- Carry out similar analysis on other proteins involved in biochemical cellular pathways
- Explore the sensitivity and selectivity of multiplexed protein biomarker detection using an antibody-based immunoassay
- Develop plasmonics-enhanced nanoprobes for DNA sensing by fabricating metallic nanostructures on fiber optic tips

Reference

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