FIBER-OPTIC MULTIPHOTON FLUORESCENCE SPECTROSCOPY FOR BIOSENSING AND *IN VIVO* FLOW CYTOMETRY

by

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To my Mom

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ABSTRACT

FIBER-OPTIC MULTIPHOTON FLUORESCENCE SPECTROSCOPY FOR BIOSENSING AND *IN VIVO* FLOW CYTOMETRY

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There is considerable interest in developing real-time diagnostic tools to detect disease signatures and therapeutic responses *in vivo*; however, tissue scattering and absorption limit the capability of traditional optical techniques for quantitative biosensing in deep tissue. To address these limitations, we have developed a fiber optic two-photon-excited fluorescence probe system to enable deep tissue quantitative measurements *in situ*. A high sensitivity double-clad fiber (DCF) probe was used to measure targeted delivery of biocompatible dendrimer nanoparticles into tumor cells. In addition to quantification of specific uptake of dendrimers in the tumor, the DCF fiber has adequate sensitivity to quantify low levels of non-specific binding of dendrimers or tissue autofluorescence. Exploiting the ability of two-photon excitation to excite multiple fluorophores, we were able to quantify few-nanomolar concentrations of different emission-wavelength antibody conjugates in mouse tumors.

To enable time-resolved spectroscopic measurements, a time-correlated single-photon counting (TCSPC) module was incorporated into the system. Fluorescence lifetime changes due to quenching of the fluophores on the dendrimer conjugates were observed. In addition, fiber optic-based two-photon fluorescence correlation spectroscopy (FCS) was demonstrated for the first time with this system. Fluorescent nanoparticles as small as 7 nm in radius were measured. By inserting the fiber probe into a flow system, we demonstrated the technique's ability to measure the flow velocity of fluorescent species. When applying the technique to measure flow cells, distinct FCS curve behaviors were observed in differently labeled cells; this may enable cell differentiation by *in situ* FCS measurements.

The minimally invasive nature of the single-fiber probe geometry is suitable for *in vivo* long-term monitoring of circulating cells, which is critical in the understanding of cancer metastasis. We used the fiber probe to implement flow cytometry *in vivo* and *in vitro*. With dual-channel detection, we conducted quantitative ratiometric measurements on the detection efficiency of dual-labeled fluorescent protein-expressing cells. In the *in vitro* studies, our system showed about one order of magnitude higher detection sensitivity for green fluorescent protein (GFP)-expressing cells in whole blood when compared to the sensitivity of the free-space detection scheme. In the *in vivo* studies, cancer cells were injected into different locations in mice, and the cell circulation dynamics were monitored. Similar detection efficiency was observed for the GFP-expressing cells. The enhanced detection sensitivity of GFP-expressing cells *in vivo* may enable the study of cancer metastasis in mouse models by fluorescence techniques.