TWO-PHOTON FLUORESCENCE MEASUREMENT OF A TARGETED NANODEVICE USING A SENSITIVE DOUBLE CLAD OPTICAL FIBER

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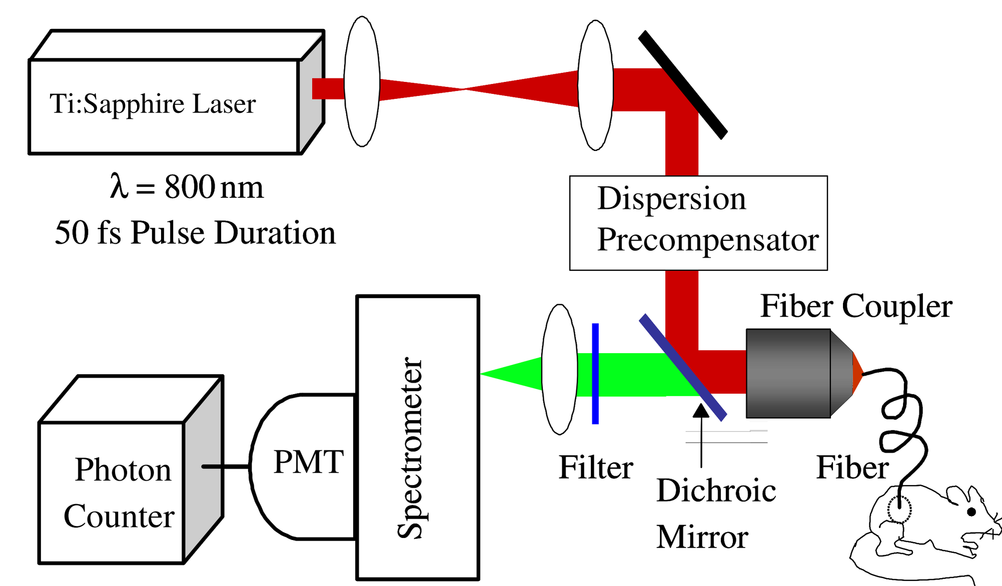
Abstract

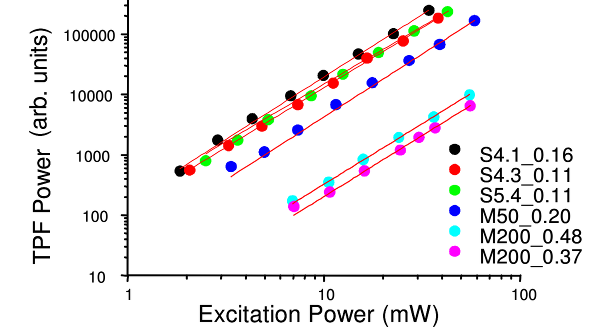
Fluorescence quantification in tissues using conventional techniques is problematic owing to the absorption and scattering of light in the tissues. Whole body fluorescence imaging techniques do not provide accurate quantitative information on the distribution of a fluorescently tagged molecule in tissues. Owing to the limited tissue penetration of light, these methods also lack sensitivity for detection of low concentrations of tissue fluorescence. Previously we have developed a two-photon optical fiber fluorescence (TPOFF) probe as a minimally invasive technique for quantifying fluorescence in solid tumors in live mice in a real-time basis (Thomas et al, *Proceedings of the SPIE*, 2006, Vol 6095). In those studies we have used a single mode optical fiber (SMF) through which femtosecond laser pulses were delivered into the tumor, which enabled us to measure low micromolar concentrations of targeted fluorescent nanoparticles. It is essential that a more sensitive TPOFF device is developed for quantification of lower levels of a targeted fluorescent agent. Here we demonstrate the biological application of a double-clad optical fiber (DCF) that can keep high excitation rate by propagating ultrashort laser pulses down an inner single mode core, while improving the collection efficiency by using a high-NA multimode outer core confined with a second clad.

The DCF does not have a hole which prevents the capillary suction of biological fluids which is a problem for the biological application of a previously described Double Clad Photonic Crystal Fiber (DCPCF, Ye, et al, *Proceedings of the SPIE*, 2005. 5700: p. 23-27). The solid DCF used has a numeric aperture of 0.46, which is smaller than that of a DCPCF. Although it does not achieve as high a collection efficiency as the DCPCF, it provides significant improvement over traditional single-clad fibers. We have compared the two-photon fluorescence detection efficiency of using the DCF vs. SMF with standard solutions of the dye 6-TAMRA (6T), and the generation 5 dendrimer (G5) nanoparticles G5-6T and G5-6T-Folic acid (G5-6T-FA). We have observed about 6-fold increase in the detection efficiency of these fluorescent agents. We then compared the targeting of G5-6T-FA in FA receptor (FAR)-expressing cells in vitro, and finally demonstrated the applicability of the DCF fiber to quantify the in vivo targeted uptake of G5-6T-FA in mice tumor expressing FAR. In summary, the DCF-TPOFF probe is an appropriate tool to quantify nanomolar levels of a targeted nanoparticle in deep tissue in vivo.

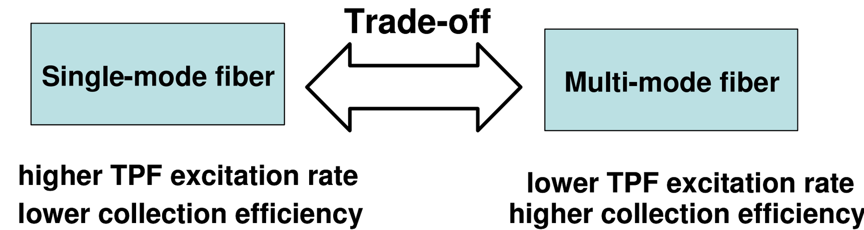
Two-Photon Optical Fiber Fluorescence (TPOFF) Biosensing



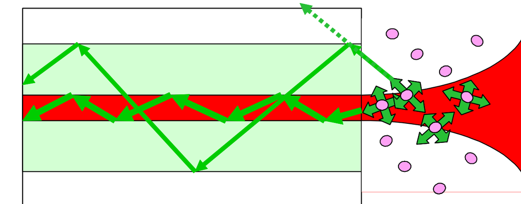
Detection Efficiency of Different Fibers



Fiber	Type	Core Diameter (μm)	Numerical Aperture (NA)	Coupling Efficiency (%)
S4.1_0.16	Single-mode	4.1	0.16	41
S4.3_0.11	Single-mode	4.3	0.11	46
S5.4_0.11	Single-mode	5.4	0.11	52
M50_0.20	Graded-index multimode	50	0.20	70
M200_0.48	Step-index multimode	200	0.48	66
M200_0.37	Step-index multimode	200	0.37	67

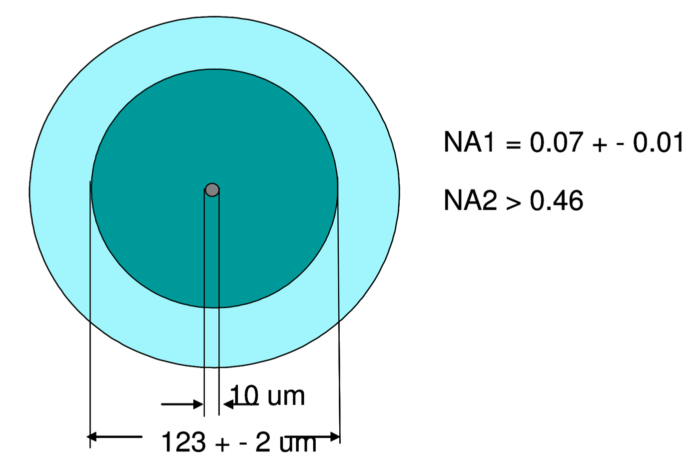


Solution: A Double- Clad Fiber



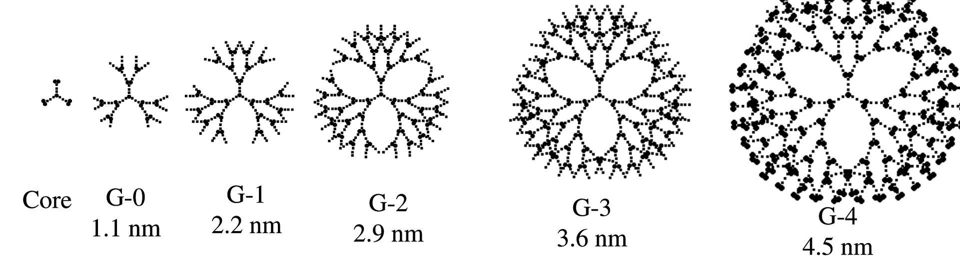
Enhancing Fiber-Optic Sensing Technique Using a Dual-Core Fiber
J. Y. Ye, T. B. Norris, M. T. Myaing, T. Thomas, J. R. Baker, Jr.
Patent filed in December, 2002.

Solid Double-Clad Fiber (DCF)

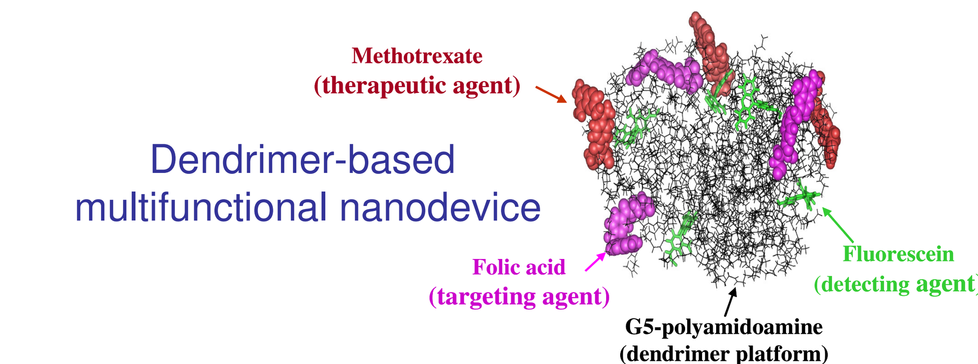


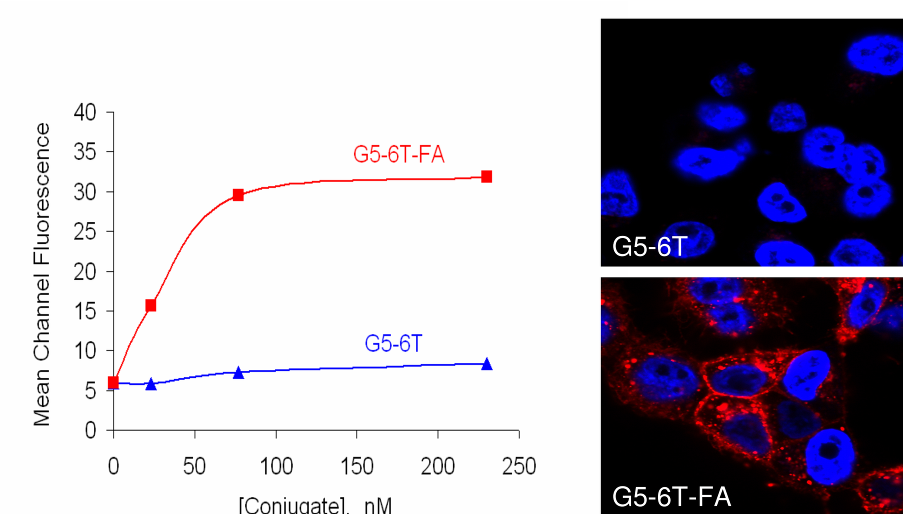
The DCF used in this study has a numerical aperture of 0.46, which is smaller than that of a double clad photonic crystal fiber we have used previously DCPCF, (Ye, et al, *Proceedings of the SPIE*, 2005. 5700: p. 23-27). Although the collection efficiency is not quite as high as for the DCPCF, it still provides significant improvement over traditional single-clad fibers.

Poly(amidoamine) (PAMAM) dendrimer

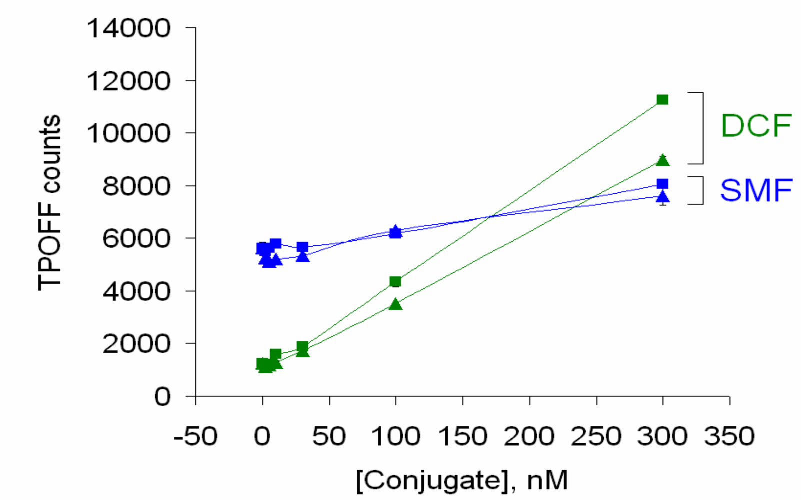


Dendrimer-based multifunctional nanodevice

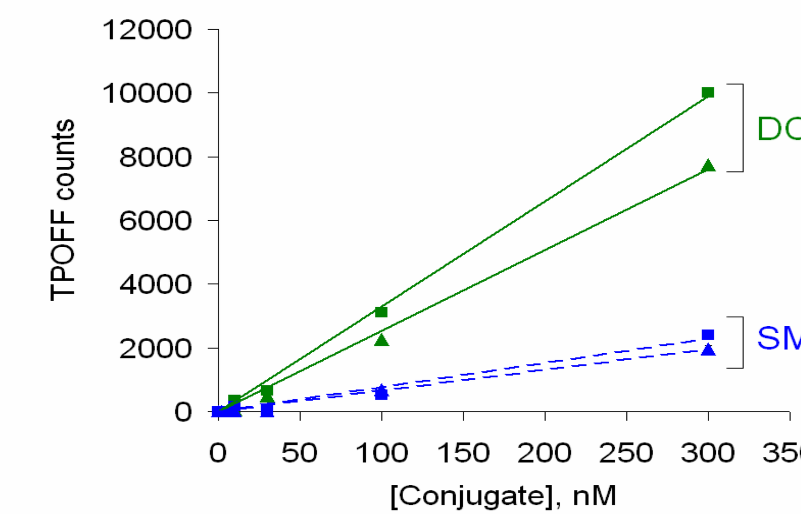




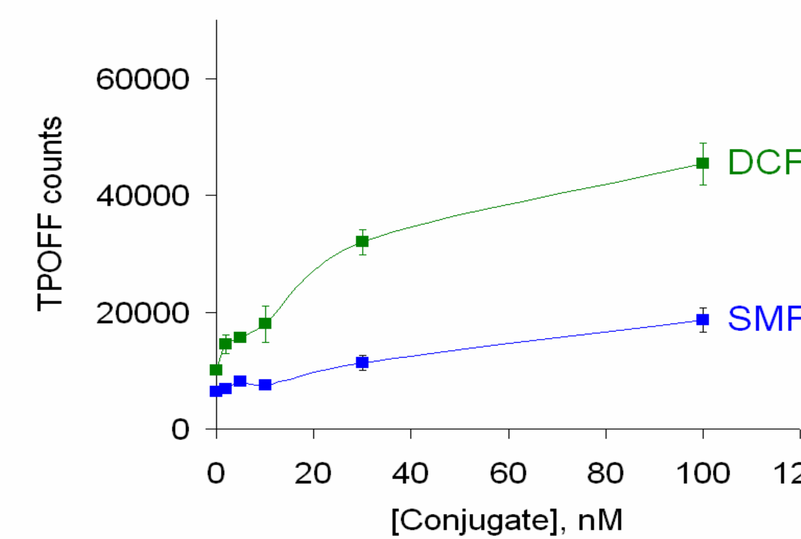
The targeted nanodevice G5-6T-FA specifically binds and internalizes into FA-receptor-expressing KB cells *in vitro*, as determined by flow cytometry and confocal microscopy. *Blue: Nuclei stained with DAPI; Red: 6-TAMRA fluorescence*



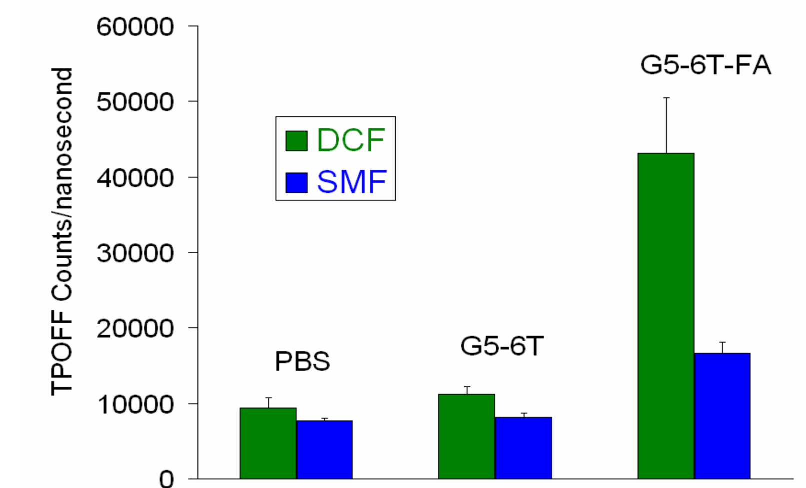
Comparison of TPOFF measurement of G5-6T (triangle) and G5-6T-FA (square) using SMF and DCF. Shown are the raw counts per nanosecond collected over 3 fluorescence life-times of 35 nanoseconds.



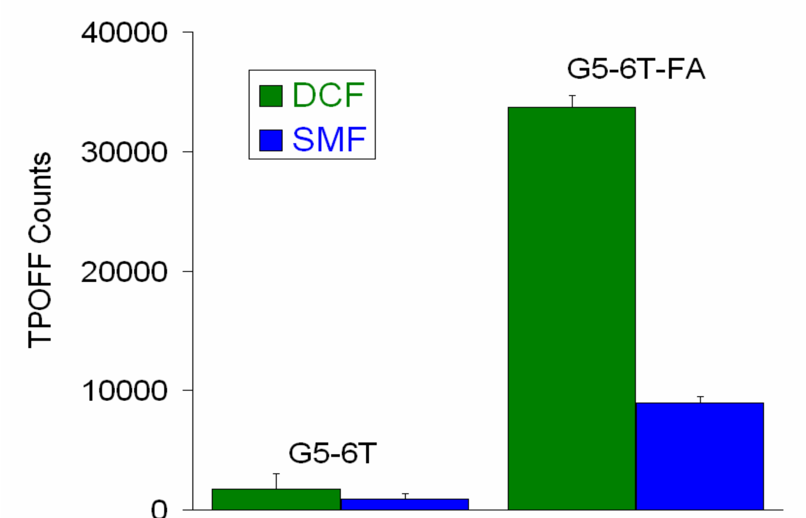
Comparison of TPOFF measurement of G5-6T (triangle) and G5-6T-FA (square) using SMF and DCF. Shown are the counts corrected for the control cell fluorescence. Other details are as shown above.



G5-6T-FA targeting of KB cells determined by SMF vs. DCF probes. KB cells in suspension were incubated with different concentrations of the conjugate for 1 h, the cells were rinsed, and the TPOFF counts were taken in the cell pellet.



In vivo tumor targeting of G5-6T-FA, determined by SMF vs. DCF probes. KB cell tumors were developed in SCID mice and were intravenously injected with the conjugates shown. After 15 h, the tumors were isolated and counts taken.



In vivo tumor targeting of G5-6T-FA, determined by SMF vs. DCF probes. Shown are the counts corrected for the fluorescence of the control tumor. Other details are as shown above.

Conclusions

This study demonstrates that the DCF-TPOFF probe has about 4-fold lower tissue detection limit in comparison to an SMF probe. The improved sensitivity of the DCF vs. SMF and the application of a small 30-guage needle make this a superior optical fiber probe technique in quantifying fluorescently tagged nanoparticles and for monitoring targeted drug delivery in deep tissues. Nanomolar quantities of multiple fluorophores with different emission wavelengths or having different lifetimes could be discerned and quantified using the technique described here. We envision that the TPOFF probe will serve as a minimally invasive diagnostic tool for screening tumor markers such as the human EGF-receptor 2 (HER2) in women with breast cancer, when used in conjunction with a fluorescent marker such as the fluorescently tagged antibody Herceptin.