A Semi-Analytic Model for Fiber-Based Fluorescence Measurements

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Abstract: A semi-analytic model is presented for calculating the fraction of fluorescent light returning to an optical fiber (which also delivers the excitation light). The model depends upon the observation that the collected light has been scattered only a few times.

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A semi-analytic model for a single optical fiber that delivers exitation light and collects fluoresence emission is presented. The amount of light collected by the fiber will depend on the properties of the fluorophore, the characteristics of the fiber (e.g., numerical aperture, diameter) and on the optical properties of the medium at the excitation and emission wavelengths. The obvious method for estimating the fluorescent light returning to the fiber is to use a Monte Carlo model.

A semi-analytic model was created to avoid long Monte Carlo simulations. A wholly empirical model was not considered because of the number of optical parameters. The semi-analytic model requires a single Monte Carlo simulation for conservative scattering to obtain the model coefficients. Absorption at the exitation and emission wavelengths is incorporated analytically into the model.

Similar semi-analytic methods have been used in the past [1], but this method is especially appropriate for the single fiber fluorescence problem because the number of scattering events is small. Pogue and Burke [2] showed that, on average, fluorescent light returning to a 200 μm fiber was characterized by an average of one scattering events for the excitation photons and less than half for the emission photons. (The scattering coefficient was 10 mm and the anisotropy was 0.9.) This observation suggests that time-consuming tracking of photons that have undergone many scattering events contributes little to the light collected by small fibers and should be avoided.

The basis for this approximation is that the relationship between the number of emission scattering events and

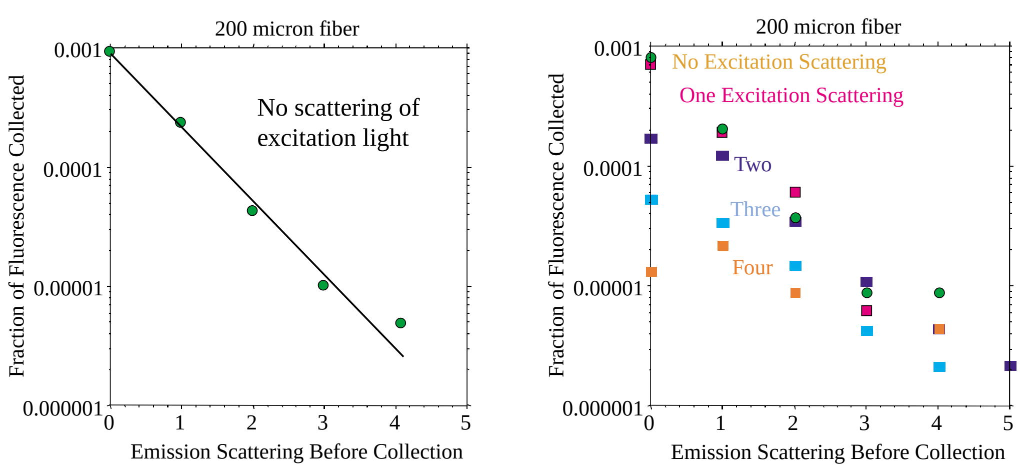

Fig. 1. The fraction of fluorescent light collected by the fiber for excitation light that has never been scattered (left). Note that the contribution to the light collected has dropped by a factor of 100 after only three emission scattering events. The graph on the right shows trends observed for various number of scattering events for the exitation photons. The scattering coefficient was 10 mm and the anisotropy was 0.9.

the collected light is very simple for a small fiber. For example, one can calculate the fraction of fluorescent light collected by a 200 μm that arises from excitation photons that was absorbed before ever being scattered. The emission photon may undergo any number of scattering events before being collected by the fiber. If one plots the fraction of emission photons that reach the fiber face as a function of the number of scattering events that the emission photon has undergone, then figure 1 (left) is obtained. Similarly, one can repeat this process for excitation photons that have scattered exactly n times, were absorbed, and then re-emitted as an emission photon. These are shown in figure 1 (right).

The semi-analytic model is uses a single Monte Carlo simulation for a specific fiber size and numerical aperture, scattering coefficients, and anisotropy coefficients. For this Monte Carlo simulation, the absorption coefficient of the medium is zero for both the excitation and emission wavelengths. The the fraction of light reaching the fiber that has undergone n excitation scatterings and k emission scatterings is given by

$$f_{nk} = A_n e^{-\beta_n k}$$

where the coefficients A_n and β_n are obtained from a linear fit to the data on a semi-log plot as shown in figure 1 (left). These coefficients are characteristic of excitation light that has been scattered n times. The total fluorescence collected by the fiber for emission light that has been scattered n times would be

$$F_n \approx \sum_{k=0}^{\infty} f_{nk}$$

when no absorption of the emission light was allowed. However, if the single scattering albedo a_m at the emission wavelength is less than one, then the the total fluorescence collected by the fiber for emission light that has been scattered n times can be approximated as

$$F_n \approx \sum_{k=0}^{\infty} a_m^k f_{nk}$$

which can be summed explictly to obtain

$$F_n \approx \frac{A_n}{1 - a_m e^{-\beta_n}}$$

The total fluorescence collected by the fiber is

$$F = \sum_{n=0}^{\infty} F_n$$

In the presence of absorption at the excitation wavelength (and therefore the excitation albedo a_x is less than one), this expression becomes

$$F \approx \sum_{n=0}^{\infty} \frac{A_n a_x^n}{1 - a_m e^{-\beta_n}}$$

Depending on the fiber size, accurate approximations of the collected fluorescence are possible using as few as four terms in this sum.

References

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2. B. W. Pogue and G. Burke, “Fiber-optic bundle design for quantitative fluorescence measurement from tissue,” Appl. Opt. **37,** 7429–7436 (1998).