Monte Carlo software can be downloaded from `http://omlc.ogi.edu/software/mc/index.html`, including a listing of the `mcfluor()` program of this chapter.

2. THE GENERAL SOLUTION

Consider a simple problem: A narrow beam of collimated light irradiates a tissue orthogonal to the tissue surface. The light penetrates the tissue and undergoes multiple scattering events that deflect the photons. Each photon initially has a trajectory pointing down into the tissue, but after numerous scattering events the photon's trajectory becomes randomized. All of the photons experience this transition from an initially directed launching to an eventually randomized trajectory. Once all photon trajectories are randomized, the net flow of light is directed along gradients in photon concentration, in which case diffusion theory is useful for modeling the light distribution. However, the Monte Carlo method is especially useful in modeling the initial transition from directed to randomized trajectories. Much of the fluorescence generation occurs during this transition. Hence, Monte Carlo simulation is an attractive method for simulating generation and escape of fluorescence in a tissue.

A generic mathematical description of the problem describes the escaping flux density of fluorescence, J_f [W/cm^2], at a position $\underline{r}$ on the tissue surface:

$$J_f(\underline{r}) = P_0 \int_{\text{volume}} T_x(\underline{r}')\varepsilon C(\underline{r}')Y T_f(\underline{r}, \underline{r}')\, dV(\underline{r}') \tag{1}$$

where

$\underline{r}'$ [cm]	is a vector that specifies the position of the incremental volume $dV(\underline{r}')$ within the tissue,
$dV(\underline{r}')$ [cm^3]	is the incremental volume at $\underline{r}'$ of the volume integration,
P_0 [W]	is the incident power of the excitation beam,
$T_x(\underline{r}')$ [cm^{-2}]	is the transport of source power at the excitation wavelength such that $P_0T_x(\underline{r}')$ yields the local fluence rate at $\underline{r}'$,
ε [cm^{-1} M^{-1}]	is the extinction coefficient of the fluorophore (using base e),
$C(\underline{r}')$ [M]	is the concentration of fluorophore at $\underline{r}'$,
Y [W/W]	is the power yield, W fluorescence per W excitation absorbed by fluorophore,
$T_f(\underline{r}, \underline{r}')$ [cm^{-2}]	is the transport of fluorescence power from $\underline{r}'$ to yield

the escaping energy density at the tissue surface at position $\underline{r}$,

$J_f(r)$ [W/cm^2] is the flux density of escaping fluorescence at the surface at position $\underline{r}$.

The transport factor T_x accounts for how the beam is launched into the tissue, e.g., as a collimated beam or a focused Gaussian beam, including consideration of any specular reflectance at the air/tissue surface and how that excitation light spreads throughout the tissue. The lumped factor εC is the absorption coefficient of the fluorophore at the excitation wavelength, defined using base e such that transmission T through a pathlength L is given as $T = \exp(-\varepsilon CL)$. Note that the literature usually reports the extinction coefficient using base 10 such that $T = 10^{-\varepsilon CL}$, so the ε of this chapter equals the literature's ε times ln(10), i.e., 2.3-fold larger. The factor Y is the power yield of W fluorescence per W excitation absorbed by fluorophore. The incremental volume of the integration, dV, has units of cm^{-3}. The product $P_0T_x\varepsilon CY dV$ has units of power [W] and serves as an isotropic point source of fluorescent power. The transport factor T_f accounts for how this fluorescent power distributes throughout the tissue and escapes at the surface, including consideration of total internal reflectance at the air/tissue surface. The final result is the escaping flux density, J_f [W/cm^2].

Similarly, the fluence rate F_f [W/cm^2] at a position $\underline{r}$ within the tissue is expressed:

$$F_f(\underline{r}) = P_0 \int_{\text{volume}} T_x(\underline{r}')\varepsilon C(\underline{r}')YT_f(\underline{r}, \underline{r}')\, dV(\underline{r}') \qquad (2)$$

where in this case $T_f(\underline{r}, \underline{r}')$ indicates the transport of local fluorescent power generated at position $\underline{r}'$ to an observation position $\underline{r}$ within the medium to yield the fluence rate $F_f(r)$ [W/cm^2]. The only difference between Eqs. (1) and (2) is that the former calculates the escape of photons from the surface whereas the latter calculates the concentration of photons at a position within the tissue. Because the units of J_f and F_f are the same, the equations look identical. Only the factor $T_f(\underline{r}, \underline{r}')$ differs between the two equations.

3. THE MONTE CARLO SOLUTION

To implement Eqs. (1) and (2) using Monte Carlo simulations, the program `mcfluor.c` uses a Monte Carlo subroutine called `mcsub()`. In the first step, `mcfluor.c` uses `mcsub()` to launch and propagate excitation photons into a medium with the optical properties of the excitation wavelength yielding the net statistical result for the transport factor $T_x(\underline{r}')$ [cm^{-2}]. The program does not specify P_0 but rather refers to P_0 as the "incident power."

Therefore, `mcsub()` returns the factor $T_x(\underline{r}')$, which can be called the fluence rate of excitation per W of incident power, $F_x(\underline{r}')$ [W/cm^2 per W of incident power] or [W/cm^2/W]. $F_x(\underline{r}')$ is the distributed excitation light that will excite fluorescence.

Next, `mcfluor.c` considers the fluorescence that is generated from a uniform background of fluorophore concentration throughout the medium. The simulation uses `mcsub()` to launch fluorescence photons into a medium with the optical properties appropriate for the fluorescence emission wavelength. The photons are launched as an isotropic point source of fluorescence from the position $\underline{r}'$. The result returned by `mcsub()` is the impulse response $T_f(\underline{r}, \underline{r}')$ [W/cm^2/W]. The impulse response is then multiplied by the fluorescent power associated with the incremental volume $dV(\underline{r}')$, which is $T_x(\underline{r}')\varepsilon CY dV(\underline{r}')$ [W per W of incident power] or [W/W]. The result, $T_x(\underline{r}')\varepsilon CY dV(\underline{r}')T_f(\underline{r}, \underline{r}')$, is the incremental contribution to $F_f(r)$ from the incremental volume $dV(\underline{r}')$. Iteratively, the program uses `mcsub()` to launch fluorescence power from each of the incremental volumes $dV(\underline{r}')$, multiply the result by the local fluorescent power in $dV(r')$, and accumulate these contributions into a final total fluence rate of fluorescence, $F_f(r)$ [W/cm^2/W]. Similarly, during these iterative calculations, the escaping flux density of fluorescence, $J_f(r)$ [W/cm^2/W], is also accumulated. These iterative accumulations are equivalent to the integration of Eqs. (1) and (2).

The subroutine `mcsub()` propagates photons in Cartesian coordinates (x, y, z); however, the results are recorded in cylindrical coordinates (z, r) because of the cylindrical symmetry of the problem. Therefore, the final result is reported as $J_f(r)$ and $F_f(z, r)$ where z is the depth position and r is the radial position of an observation point. Throughout calculations, such as when launching a photon, a radial position r is sometimes assigned to a position x with y = 0, which is appropriate since the model has cylindrical symmetry.

Finally, `mcfluor.c` considers the fluorescence that is generated from a local small heterogeneity of extra fluorophore. The heterogeneity is characterized as an extra fluorescence in a small volume V_h at a specific position $\underline{r}_h = (x_h, y_h, z_h)$ with ε, C, and Y given values of ε_h, C_h, and Y_h. The program determines the fluorescent power source to be $T_x(\underline{r}_h)\varepsilon_h C_h Y_h V_h$ [W/W]. The impulse response is obtained by `mcsub()` launching a fluorescent isotropic point source at $\underline{r}' = (0, 0, z_h)$ to yield $T_f(\underline{r}', \underline{r}'')$, where $\underline{r}''$ is the position of an observation point relative to the fluorescent source. The points of observation are placed along the x axis with y = 0. The $\underline{r}''$ equals the line from the heterogeneity at $\underline{r}_h$ to some point of observation $\underline{r}$ along the x axis (with y = 0). Let this line be called $\underline{r}_{ho}$. Then the contribution of the fluorescent heterogeneity to fluorescence observed at a position $\underline{r}$ is determined:

$$F_f(\underline{r}) = T_x(\underline{r}_h)\varepsilon_h C_h Y_h V_h T_f(\underline{r}', \underline{r}_{h0})$$

where $T_f(\underline{r}', \underline{r}_{h0})$ is the impulse response F(z, r) [W/cm^2/W] returned by `mcsub()` after launching an isotropic point source at (0, 0, z_h). Without an underline, z and r denote depth and radial positions, respectively.

Similarly, the contribution of the fluorescent heterogeneity to escaping flux density observed at a position r on the surface along the x axis (y = 0) is determined:

$$J_f(r) = T_x(\underline{r}_h)\varepsilon_h C_h Y_h V_h(\underline{r}', \underline{r}_{h0})$$

where $T_f(r', r_{h0})$ is the J(r) returned by `mcsub()` after launching an isotropic point source at (0, 0, z_h).

The program `mcfluor.c` illustrates the calculation of fluorescence from a single small heterogeneity that is chosen to have a spherical shape. The program can be adapted to consider the contribution from several such heterogeneities. Superposition of many such heterogeneities can yield the response to a larger irregular region of extra fluorescence above the background fluorescence. Keep in mind that the heterogeneity problem breaks the cylindrical symmetry. Another caveat is that the added absorption of this fluorophore should not significantly increase the overall absorption coefficients at either the excitation or the emission wavelength. Otherwise, one no longer has a homogeneous problem for propagation of excitation and fluorescent emission and the user may need to modify `mcsub()` to consider local absorption of the heterogeneity.

4. SAMPLE SIMULATIONS

To illustrate the use of the program `mcfluor.c`, three example simulations are shown (Fig. 1). The first example is the launching of an isotropic point source of excitation light from a depth of 0.5 cm. The optical properties at the excitation and fluorescent wavelengths are as follows:

Property	Unit	Excitation	Fluorescence
Absorption coefficient	μ_a	1 cm^{-1}	0.2 cm^{-1}
Scattering coefficient	μ_s	10 cm^{-1}	5 cm^{-1}
Anisotropy	g	0.90	0.90

The fluorophore properties are εC = 0.1 cm^{-1} and Y = 1. The refractive index of the medium is n_1 = 1.33 with an external air boundary (n_2 = 1.00).