Monte Carlo Modeling of Near-infrared Fluorescence Photon Migration in Breast Tissue for Tumor Prediction

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Abstract Breast cancer is one of the most common types of cancer in Japanese women. To address the low spatial resolution challenges associated with mammography and ultrasonography, we focused on the potential of using fluorescence to observe cellular and subcellular structures. Light scattering in living tissue causes a decrease in resolution in in vivo imaging. However, scattering in near-infrared region is weaker than that in the visible region. Therefore, it is essential to investigate the behavior of excitation and emission photons in near-infrared fluorescence within tissues, which could be applied in the detection of breast cancer. We modified our previous multi-layered fluorescence Monte Carlo model of in vivo neuroimaging using quantum dots as the first step for the detection of early-stage breast tumor using both visible and near-infrared light, and developed a model containing skin, breast tissue, and tumor. In the present study, fluorophore concentration and quantum yield parameters were set appropriately based on the mechanism of fluorescence onset. When the depths and sizes of a fluorescent tumor embedded in the breast tissue model were varied, excitation and emission fluence, in addition to intensity were examined from the breast surface. In contrast to visible fluorescence (Ex 488/Em 520), Monte Carlo simulation for breast cancer using near-infrared fluorescence (Ex 780/Em 820) could be used to detect a tumor 1.0 cm in diameter at a depth of 1.0 cm.

Keywords: Monte Carlo simulation, near infrared, scattering, absorption, breast cancer.

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1. Introduction

Breast cancer is one of the most common cancers among women aged between 15 and 60 years in Japan, and the number of deaths due to breast cancer has been increasing over the year. If tumors smaller than 2 cm can be detected, breast conservation therapies could be selected as treatment option. Detection of early-stage breast cancer tumors smaller than a few millimeters are challenging not only by mammography but also by ultrasonography, due to their low spatial resolution [1]. On the other hand, fluorescence imaging technologies provide high resolution at cellular and subcellular levels, but resolution inevitably decreases in in vivo imaging due to light scattering by living tissues. Nevertheless, scattering in the near-infrared region is weaker than in the visible region [2].

In addition to blue dyes such as Patent Blue, the near-infrared fluorescent dye Indocyanine Green (ICG) has been used in sentinel lymph node biopsy. Identification of sentinel lymph nodes was improved using a longer wavelength range [3, 4]. Still, success depends on skills and experience because of diverse depths and sizes of the nodes. Furthermore, in lymph node metastasis, axillary lymph node dissection is inevitable. Small breast tumor that does not metastasize to the lymph nodes is expected to be detected by fluorescence without biopsy. Using fluorescence between visible and near-infrared regions, we investigated the behavior of excitation and emission photons in breast tissues by numerical analysis and examined the possibility of detecting early breast cancer. In general, the photon diffusion equation (PDE) is numerically solved to investigate light propagation in biological tissue [5, 6]. The use of analytical solutions of...
PDE is limited to simple models.

In the Monte Carlo simulation of visible light transmission for breast cancer detection, the tissue model has been defined as a monolayer slab with scattering and absorption coefficient parameters typical of breast tissue [7]. The model is a simulation equivalent to the scanning arrangement of X-ray mammography, in which images are acquired from a breast 4–5 cm thick compressed between parallel plates. After embedding a spherical object with different absorption coefficients in the slab, the imaging contrast is examined. Since actual breast tissue has more complex structures including skin, subcutaneous fat, and mammary glands, the monolayer slab model may not always be accurate [1].

We previously developed a multi-layered fluorescence Monte Carlo model for in vivo neuroimaging using quantum dots [8, 9] as the first step in the detection of early-stage breast cancer, and consequently developed a model containing skin, breast tissue and tumor using visible and near-infrared fluorescence. In the present study, we set appropriate fluorophore concentration and quantum yield parameters based on the mechanism of fluorescence onset [10]. Instead of quantum dots, we used quantum yield of organic fluorescent dyes for cancer diagnosis. We varied the depth and size of the fluorescent tumor embedded in the breast tissue model, and examined excitation and emission fluence in addition to intensity from the breast surface. Compared with visible fluorescence, near-infrared fluorescence may be more suitable for detecting more deeply seated and smaller tumors.

2. Simulation

2.1 Tumor model and optical parameters

As shown in Fig. 1, the model composed of 0.2-cm thick skin on 1.8-cm thick breast tissue based on the anatomical properties of the breast and description in the literature [1]. The model was expected to simulate the upper outer region of breast, which is a region associated with high risk of developing breast cancer. A fluorescent tumor was embedded in the model at a depth of 0–1.0 cm from the skin surface. The diameter of the tumor was 0.2–1.0 cm. In order to investigate the relationship between the radius of detection area and the detected fluorescence intensity, the detection area was divided into a circular area indicated by the dark gray zone and an annulus area indicated by the light gray zone.

To examine the effect of tumor size and depth on fluorescence imaging, excitation and emission photons were tracked in the model using Monte Carlo based on optical parameters including scattering coefficient, absorption coefficient and refractive index of skin and subcutaneous fat, according to previous reports [11, 12] (Table 1). The forward scattering property of the tissue was very high from the visible region to the near-infrared region, and the wavelength dependency was low [11]. We used an anisotropic parameter, \( g = 0.9 \), and two fluorescent dyes: fluorescein (excitation max (Ex) 488 nm, emission max (Em) 520 nm, quantum yield (QY) 0.95, concentration (C) 0.75 mM, absorption coefficient (\( \varepsilon_C \))...
12 cm\(^{-1}\)} and ICG (Ex 780/Em 820, QY 0.09, C 0.2 mM, εC 20 cm\(^{-1}\)) [2, 3, 13–18]. The former is used in retinal angiography and the latter is used in sentinel lymph node biopsy. Each QY was the quantum yield in aqueous solution [13, 14]. The fluorescent dye concentrations were those used clinically [2, 3]. Such considerations for clinical applications may be beneficial in the future when antibodies are labeled by fluorescent dyes that can be recognized by tumor markers such as EGFR (epidermal growth factor receptor), HER2 (human epidermal growth factor receptor type2), and VEGFR (vascular endothelial growth factor receptor).

2.2 Monte Carlo simulation
Photon movement in the present study was based on a three-dimensional random walk model [19]. Each path length of the random walk model corresponded to the distance traveled by a photon from an interaction site between photon and tissue due to scattering and absorption to the next site. The path length was calculated using a random number and total attenuation coefficient \(\mu_t\), which is the sum of scattering coefficient \(\mu_s\) and absorption coefficient \(\mu_a\). During movement of the photon to the next site, the original weight of the photon was reduced by \(\mu_a/\mu_t\). We used pseudo-random numbers generated using the Mersenne twister method [20]. The direction to the next site was set using the angle of scattering from the original direction of propagation to the new one. Other than the first step of generation of fluorescence with isotropic emission, an isotropic azimuth and a zenith angle with a highly forward scattering property toward the next interaction site were generated using different random numbers. The forward scattering property of tissue was set based on the Henyey-Greenstein function [21]. When photons crossed the boundary between two layers, they were reflected or transmitted based on Fresnel reflectance calculated using refractive indices of each layer [22]. Random paths for photons were computed until the weight of each photon was absorbed to less than a threshold or the photon left the medium.

2.3 Monte Carlo fluorescence model
The software for Monte Carlo modeling of fluorescence was developed using C programming language with Microsoft Visual Studio. The model was largely consistent with the concepts outlined by Welch et al [23]. A million photons at the excitation wavelength with initial weight of unity were input into the model along the light axis from the origin of space coordinates. Each photon was tracked during random walk based on the optical parameters of tissues (Table 1). Excitation photon propagation was displayed as internal photon distribution using fluence, which scores the photon weight absorbed within the grid elements (r, z) based on the absorption coefficients of skin, subcutaneous fat, tumor, and fluorescent dye. The step size was 0.02 cm; therefore, each element occupied 0.02\(^3\) cm\(^3\) [9]. Here, fluence is the weight of photons that passed per unit area. In short, it is the amount of energy that passed per unit area (W/cm\(^2\)). When photons crossed the lower boundary of the 2 cm breast model, the absorption of light outside the boundary was not reflected in the fluence [24]. We considered that the photons did not return inside the breast model due to strong scattering and absorption of living tissue. Therefore, these photons were excluded from calculation. After the completion of tracking of all the excitation photons, a million photons at emission wavelength were generated at points determined randomly within the tumor. There was insufficient computer resources to increase the number of photons as the tumor became bigger. In addition, as the tumor became smaller, reducing the number of photons lowered calculation accuracy. Therefore, the initial weight of photons was adjusted according to the size of the tumor. In the first step of the generation, both azimuth and zenith angles toward the subsequent interaction site were set to be isotropic using different random numbers. Thereafter, isotropic azimuth and zenith angles with highly forward scattering properties toward the next interaction site were generated. After completion of tracking of all emission photons, the data of fluence were multiplied by the product of excitation fluence of the tumor center, fluorescent concentration and its QY, which gave fluorescence fluence [9, 10]. Fluorescence intensity was the sum of photon weight detected on the skin surface of the bin range per unit area, W/cm\(^2\) (Fig. 1) [9].

3. Results and Discussion
3.1 Effect of tumor depth and size
To examine the potential of detecting early-stage breast cancer, we investigated the effects of the depth of the fluorescent cancer on fluorescence intensity in the near-infrared region (Ex 780 Em 820) on the breast surface. The intensity per unit area was scored based on the sum of the weight of fluorescence photons from the tumor (1.0 cm in diameter along the axis) embedded at depths of 0–1.2 cm in the breast model (Fig. 1). In order to investigate the relationship between tumor depth and detected fluorescence intensity, the intensity was detected in a circular area with a radius of 0.4 cm including the axis. When the flat surface of the breast was tangent to the upper tumor surface (z = 0), the intensity on the axis was 4.3 \(\times\) 10\(^{-2}\) W/cm\(^2\) (Fig. 2A). At a depth of 0.2 cm, the intensity decreased to 2.2 \(\times\) 10\(^{-3}\) W/cm\(^2\), which was equivalent to 5.1% of the intensity at z = 0. At depths of
At a depth of 1.0 cm, the intensity was weak at $2.7 \times 10^{-8}$ W/cm$^2$. In the case of tumor diameter of 0.8 cm, emission photons with 0.51 as an initial weight yielded a fluorescence intensity of $1.6 \times 10^{-8}$ W/cm$^2$. For diameters of 0.6 and 0.4 cm, the intensities were $7.4 \times 10^{-9}$ W/cm$^2$ due to initial weight of 0.22 and $2.2 \times 10^{-9}$ W/cm$^2$ due to initial weight of 0.064, respectively.

At a diameter of 0.2 cm, almost no fluorescence intensity was detected. Although weaker intensity was obtained from smaller tumors, the size had no effect on the profile of fluorescence intensity detected away from the axis, because of matching of the normalized profiles. In addition, we confirmed that when the detection area shifted from the circular area including the axis, the detected fluorescence intensity became very weak. Therefore, it is essential to set the detection area carefully. When the tumor was labeled with a near-infrared fluorescent dye such as ICG, fluorescence was detected from a tumor with a diameter of 1.0 cm at a depth of 1.0 cm. However, smaller tumors would require greater care due to weaker fluorescence.

**3.2 Comparison with imaging in the visible range**

To examine the significance of near-infrared fluorescence for detecting early breast cancer, fluence due to excitation and emission photons in visible fluorescence was also examined. Fluence is an indicator of photon distribution within the model and the probability of photons being absorbed by the grid element. Here, the excitation light was incident from $r = 0, z = 0$ (Fig. 3A, B), and the fluorescence light source was arranged as a fluorescent tumor with a diameter of 1 cm centered at $r = 0, z = 1.5$ (Fig. 3C, D). As shown in Fig. 3, excitation photons at 488 nm (A) propagated in a region narrower than the region propagated by excitation photons at 780 nm (B). The region that photons propagated is restricted by absorption and scattering, and the absorption and scattering coefficients at 488 nm are larger than those at 780 nm (Table 1). Notably, almost no fluence was observed at 488 nm at a depth below 1.0 cm, even though a fluorescent tumor with a diameter of 1.0 cm was localized in a deeper area of the circle, which is indicated by dotted lines (A). Therefore, no fluorophores within the tumor were excited (A) and no emission photons (C) were absorbed in the visible range. In contrast, near-infrared excitation photons at 780 nm propagated in the model extensively and the weights were partially absorbed (B).
Compared with the region near the origin where photons entered, fluence in the tumor was weak and the difference in fluence among grids within the tumor was also minor (B). Therefore, as the first step to establish the breast cancer model, it may be appropriate to generate emission photons with a constant weight at a randomly determined position, but generation of photons with an initial weight dependent on the position is necessary in the subsequent step. As illustrated in Fig. 3D, each photon that emitted from the fluorescent tumor propagated isotropically, and part of the weight was absorbed. The fluence map suggested that emission photons reached the surface of the breast model. Fluorescent photons emitted from a fluorescent tumor (C, D) were monitored on the breast surface. In contrast to fluorescence at 820 nm (F), no fluorescence at 520 nm (E) was shown. Then, we adjusted the diameter of the fluorescent tumor to evaluate the relationship between tumor size and detection intensity. When a fluorescent tumor was considered as a point light source as shown in a previous study [9], there was no heterogeneity within the tumor. In the present study, we focused on the effect of tumor size. In order to simplify the calculation, the heterogeneity due to tumor size was ignored, although there may be an excitation gradient within a larger tumor. In the future study, we plan to subdivide the tumor by using a 3D Monte Carlo model [25] to determine the excitation intensity at each point. This will allow the calculation of fluorescence fluence reflecting the tumor excitation gradient.

The Monte Carlo simulation for the breast tumor using near-infrared fluorescence (Ex 780/Em 820) suggested the possibility of detecting tumors 1.0 cm in diameter at a depth of 1.0 cm from the surface. From a spatial resolution perspective, the measurement may not be improved considerably compared with the conventionally used X-ray mammography and ultrasonography tools [1]. However, the advantage of this fluorescence imaging for breast cancer is that molecular imaging may be able to distinguish between benign and malignant tumors. In addition, weak fluorescence in the near-infrared region was detected from tumors in size range of 0.4 to 0.6 cm diameter at a depth of 1.0 cm. This finding leads us to expect clear detection of tumors a few millimeters in diameter using near-infrared fluorescence with wavelengths longer than 820 nm. However, appropriate fluorescence dyes have not yet been used in the clinical settings. For this wavelength range, although quantum dots have been developed, use in human body is not approved because of toxicity [26]. To date, only a few organic fluorescent dyes in the above-mentioned wavelength range have been reported [27]. Clarifying their properties such as QY would facilitate the detection of early-stage breast cancer.

Conflict of Interest
We have no conflict of interest with any company or commercial organization.

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