Piecewise Weighted Tikhonov Regularization for Reconstructing Fluorophore Distribution in Tissue using Mesoscopic Epifluorescence Tomography

Tuo Zhou,* # Takehiro Ando,* Hongen Liao,* Etsuko Kobayashi,* Ichiro Sakuma*

Abstract The technique of mesoscopic epifluorescence tomography (MEFT) has been reported previously. Although it allows three-dimensional (3D) imaging of the concentration distribution of fluorophore reporters embedded in tissue in reflectance geometry with a resolution of hundreds of micrometers, reconstruction accuracy is unsatisfactory. In this study, a novel regularization method is proposed to improve the accuracy. The proposed method is a derivation of Tikhonov regularization but inherits the iterative reweighted nature of sparsity regularization. First, Tikhonov regularization is utilized to generate an initial estimation. Then, a weight matrix is generated on the initial estimation and then coupled to the regularization objective function for a new estimation. The new estimation leads to a new weight matrix that begins iteration. The weight is decided in a piecewise manner: a high but constant weight is set to the region of interest (ROI) where the fluorophore is highly likely to exist, while lower weights are set to background regions. The values in the ROI are thus enhanced to a similar degree in each iteration while those in the background regions are suppressed. We constructed a MEFT system and conducted a series of numerical simulations and phantom experiments to evaluate the performance of the proposed method in comparison with several general regularization methods. Our results showed that application of our method produced reconstructed distribution with more accurate values (concentrations) and a clearer boundary compared with Tikhonov regularization and LSQR algorithm (used in previous report). Moreover, due to the constant weight set for the ROI, our proposed method preserves local smoothness and completeness of actual fluorophore distribution, for which sparsity regularization is inadequate.

Keywords: mesoscopic epifluorescence tomography, Tikhonov regularization, sparsity regularization, piecewise-ly weighted, iterative reweighted algorithm.

1. Introduction

In recent years, significant development has been made in the field of fluorescent reporters that allow in vivo examination of the cellular biochemical process and noninvasive localization of disease foci. Because of this development, fluorescence molecular tomography (FMT) has attracted extensive attention. FMT allows three-dimensional (3D) imaging of fluorophore (concentration) distribution in small animal models or in local human tissues [1]. Conventional FMT is always implemented in transmission geometry, with a large amount of source-detector fiber pairs set around the object examined [2–4]. However, applications of FMT of this type are restricted to small animal or breast imaging. As a novel derivation of FMT, mesoscopic epifluorescence tomography (MEFT) [5], is capable of reconstructing fluorophore biodistribution in reflectance geometry, with resolution of several hundred micrometers and allows detection of up to a depth of several mm. In this technique, laser beam scanning and data acquisition by a CCD camera replace the complicated and bulky source-detector pair system, providing a dense arrangement of detectors and sources. Furthermore, reflectance geometry has the potential in imaging brain cortex (during cranial surgery), skin and abdominal cavity, from which collection of transmitted light is difficult. It is also easy to combine MEFT and fluorescence microscopy for assisting with intraoperative diagnoses. However, according to the results of a previous report [5], reconstruction accuracy starts to deteriorate when the fluorophore exceeds 700 μm. We postulate that this inaccuracy is partly owing to the application of an inappropriate reconstruction method.

The reconstruction of fluorophore biodistribution is a typical inverse problem, which estimates the fluorophore distribution $m$ from acquired fluorescence measurements $d$ based on the sensitivity matrix $G$ of light propagation in tissue. It usually involves a data fitting process to minimize the least squares of $Gm−d$. However, the process is difficult because it is often extremely sensitive to random noise in measurements. In the worst case, the inverse solution may just be a noise amplifier and physically meaningless. Therefore, fitting accuracy is
often sacrificed in exchange for solution stability, which is called regularization\[6\].

In our previous study, we investigated the performance of several popular regularization methods in the context of MEFIT, such as Tikhonov regularization, sparsity regularization, and total variation regularization, by conducting a series of numerical simulation and phantom experiments. Although the widely used Tikhonov regularization[7], provided a moderately satisfactory solution, we sought to develop a better method that could incorporate some priori knowledge into the process of reconstruction. We noticed the fact that most fluorescent reporters are designed to merely accumulate in specific regions such as cancerous cells, and they rarely enter normal tissues. Therefore, the actual fluorophore distribution is restricted to local regions within tissue, especially in the case of early cancer. Sparsity regularization[8] was thus tested because it tends to generate a locally distributed result. Unfortunately, sparsity regularization always compresses fluorophore distribution to a few points. Although this method helped to localize the center of fluorophore distribution, it results in low image quality and underestimates the fluorophore volume. Finally, total variation (TV) regularization[9] is considered to generate a solution with smooth local regions and sharp boundaries. However, our simulation result showed that these features can only be ensured in the case of low random noise (data not shown). In fact, some studies focus on combined sparsity and TV regularization. However, this method leads to more than three constraints in the objective function and consequently optimization is extremely complex and difficult[10].

In this study, we propose a novel regularization method, piecewise weighted Tikhonov regularization (PWTR), which is a derivation of Tikhonov regularization but inherits the iterative reweighted nature of sparsity regularization. We herein introduce the concept of piecewise weighting and verify its effectiveness using results obtained from simulation and phantom experimentation.

2. Methods

2.1 Inverse problem

As mentioned before, the objective of FMT is to estimate fluorophore distribution \( m \) from a linear system \( Gm = d \), where \( G \) is the sensitivity matrix and \( d \) is the observed fluorescence measurement. Note that although both \( m \) and \( d \) are originally 3D matrices, for solving inverse problem they are always reshaped into one-dimensional vectors. The objective function of a regularization method always includes a penalty term \( f(m) \) in addition to the data fitting term (Eq. 1).

\[
\min_{m} ||Gm - d||^2 + \alpha f(m) \tag{1}
\]

where \( \alpha > 0 \) is a regularization parameter and can be usually determined using the L curve criterion[11].

Additionally, because a negative fluorophore concentration is physically impossible, we added a nonnegative constraint to all the subsequent objective functions by replacing all negative reconstructed values to zero.

2.1.1 Tikhonov regularization

Tikhonov regularization (zero-order) is the most widely used method for solving inverse problems. Tikhonov regularization has an object function with a norm 2 penalty term (Eq. 2).

\[
\min_{m} ||Gm - d||^2 + \alpha m^Tm \tag{2}
\]

Equation 2 is solved easily by finding its zero gradient point as shown in Eq. 3 and Eq. 4. This method favors a lower norm 2 term and thus tends to generate a smooth inverse solution[12].

\[
\nabla ||Gm - d||^2 + \alpha m = 0 \tag{3}
\]

\[
m = (G^T G + \alpha I)^{-1} G^T d \tag{4}
\]

where \( I \) is the identity matrix.

2.1.2 PWTR

The basic idea of PWTR is to (1) generate an initial estimation using Eq. 4 (Tikhonov regularization), (2) build a weight matrix \( W \), according to the initial estimation (Eq. 5 and Eq. 6), (3) couple \( W \) into regularization (Eq. 8), and (4) produce a new weight matrix from the new estimation. The third and fourth steps iterate until the iterative condition breaks. ROI herein was considered as the region where the fluorophore is highly likely to exist, while voxels beyond ROI were treated as background. ROI was determined by an empirical threshold (Eq. 7). Weight in the ROI was set as a relatively high constant than that outside ROI. As a result, the data in ROI were enhanced to a similar degree in each iteration, while those in the background were suppressed.

For a given initial estimation \( m \) from Eq. 4, we first normalized it with its maxima to obtain a normalized estimation \( m' \). \( W \) was then determined by the following equations:

\[
W = \begin{bmatrix}
\frac{1}{w_1} & 0 & \cdots & 0 \\
0 & \frac{1}{w_2} & \cdots & 0 \\
\vdots & \vdots & \ddots & \vdots \\
0 & 0 & \cdots & \frac{1}{w_n}
\end{bmatrix} \tag{5}
\]

\[
w_i = \begin{cases}
\beta & m'(i) > \beta \\
\beta \geq m'(i) > \epsilon \\
\epsilon & m'(i) \leq \epsilon
\end{cases} \tag{6}
\]

where \( w_i \) is the weight for \( i \)th voxel, \( n \) is the number of elements in \( m \), \( m'(i) \) is the value of the \( i \)th element in \( m' \). \( \epsilon \) is a very small positive value. To avoid dividing a zero or very small non-zero value in Eq. 5, the weight of a voxel with a value smaller than \( \epsilon \) was set as \( \epsilon \). The threshold \( \beta \) was determined by an empirical equation (Eq. 7).

\[
\beta = 0.5 \overline{m}_{10\%} \tag{7}
\]

where \( \overline{m}_{10\%} \) indicates the elements of \( m' \) that have values larger than 10% of the maxima, while \( \overline{m}_{10\%} \) is the mean of those elements. The weight matrix was coupled to Eq. 4.
by insertion after the regularization parameter (Eq. 8).

\[ m = (G^T G + \alpha W)^{-1} G^T d \]  

(8)

Because of the piecewise nature of Eq. 6, we thus referred to this proposed method as piecewise weighted Tikhonov regularization. The new estimation from Eq. 8 is subsequently employed to generate a new weight matrix in the same manner for application in the next iteration. Iteration stops when the relative difference between the results of neighboring iterations \( \frac{\|m_{k+1} - m_k\|_2}{\|m_k\|_2} \) is smaller than 5%.

2.1.3 Sparsity regularization

There is a norm 1 penalty term in the objective function of sparsity regularization (Eq. 9). Previous study proved that this norm 1 term tends to minimize the number of non-zero value in the inverse solution[13], thus resulting in a locally limited distribution.

\[ \text{min} \|Gm - d\|_2^2 + \alpha \|m\|_1 \]  

(9)

Due to the nondifferentiability of \( \|m\|_1 \), Eq. 9 cannot be solved easily like Eq. 4 or Eq. 8. We used an iteratively reweighted least squares algorithm, with the same flow as that of the method we proposed. An initial estimation was generated using Eq. 4, upon which a weight matrix was determined. The weight matrix can be integrated in the regularization as Eq. 8. Subsequently iteration begins. The main difference between sparsity regularization and PWTR is the method to determine the weight \( w_0 \). In this case, the absolute value of the initial estimation \( m \) was used directly as the weight of the correspondent voxel as shown in Eq. 10. For comparison, schematic maps of Eq. 5 and Eq. 10 (the first quadrant) are shown in Fig. 1.

\[ w_0 = \begin{cases} \frac{\|m(i)\|}{\|m(i')\|} & \text{if } m(i) > \varepsilon \\ \frac{\|m(i')\|}{\|m(i)\|} & \text{if } m(i') > \varepsilon \\ \varepsilon & \text{else} \end{cases} \]  

(10)

2.1.4 LSQR algorithm

The LSQR algorithm was used in a previous report[5]. It is also an iterative method designed for large and sparse sensitivity matrices. Its objective function is just the least square term \( \text{min} \|Gm - d\|_2^2 \). However because of the bidiagonalization procedure of Golub and Kahan in this algorithm, the least square solution can be stabilized[14].

2.2 MEFT system

A MEFT system was constructed as shown in Fig. 2.

![Fig. 1](https://example.com/fig1.png)

Fig. 1 Weighting of PWRT (a) and sparsity regularization (b).

![Fig. 2](https://example.com/fig2.png)

Fig. 2 Setup of MEFT.

A solid laser (Sacher Lasertechnik GmbH, Germany) emitting a beam of 790 nm wavelength was used as the light source. The laser beam was first coupled to a single mode fiber (Thorlabs, USA) and, collimated using an aspheric FC collimator (Thorlabs). It finally became a Gaussian beam with a diameter of 0.8 mm and an output power of 0.2 mW. The laser beam was reflected by a mirror and then a dichroic mirror (801 nm, Edmund GmbH, Germany). The beam scanned over samples using motorized stages. The motorized XY stage (Sigma, Japan) had travel ranges of 30 mm. Another XY stage (Sigma, Japan) supported the sample and facilitated fine adjustments for the sample position. A tank and a glass capillary tube (Ф 1 mm) were used as containers for liquid sample and fluorophore, respectively.

Emitted fluorescence was transmitted through the dichroic mirror and was collected by a 10-bit monochrome EMCCD camera (Flovel, Japan) with high sensitivity. A notch filter (785 nm, Thorlabs) and a bandpass filter (837 nm/30 nm) were utilized to reduce the amount of excitation light detected. The camera was equipped with a 12 mm/F 1.8 lens. Owing to the large visual angle of the lens, fluorescence data existed over only a small region of the images. Each pixel in the region can be seen as a virtual fluorescence detector. However, to reduce computation cost, we usually chose to down size the images.

2.3 Forward problem

Sensitivity matrix \( G(r_s, r_d, r) \) of light propagation in tissue describes the amount of fluorescence detected by the detector \( r_d \) when a fluorophore of unity concentration localizes at the voxel \( r \) and an excitation beam incidents on \( r_s \). With prior knowledge of the tissue’s optical properties, this sensitivity matrix can be approximated by the diffusion equation or simulated by the Monte Carlo method. The former is very fast, but inaccurate in the region near the light source [15]. The latter is more
accurate but computationally expensive[16]. Because the side length of the actual observation area in MEFT is usually several millimeters, while the tissue’s scattering length at the near infra-red wavelength is in the order of 1 mm, many virtual detectors are not far from the incident source[17]. We thus selected the Monte Carlo method in this study.

The simulations were implemented in a cylindrical media 20 mm in depth and 50 mm in radius, with 0.1 mm spatial resolution in all directions. We set tissue-like optical coefficients (listed in section 3.3) for the simulated medium. For each simulation, 10⁶ photons were launched. Our Monte Carlo code was an adaptation of MCML[16] and Matlab code from Dr. Alerstam[18]. To reduce the computation cost of Monte Carlo simulation, an accelerated Monte Carlo Model was applied[19].

Figure 3 shows the steps in calculating the sensitivity matrix $G$. We first used a Monte Carlo simulation to calculate the fluence rate of excitation light (an infinitely narrow photon beam) perpendicularly incident on the medium. The fluence rate distribution was convolved with the beam profile of the actual laser beam which was measured by a beam profiler. The convoluted matrix $G_{rr}(r, d)$ was a function of radius and depth, describing the distribution of excitation light in the medium. Then, another independent simulation was performed for sensitivity matrix $G_{od}(r, d)$ of a fluorescent source embedded in the medium. Here the reciprocity principle for photon paths was applied. We set a light source on the surface of medium and calculated the fluence distribution of photons in the medium, rather than launching photons at various depths within the medium and counting photons emitting out of the surface. The solid angle of the fluorescent source was set as equal to the collect angle of the EMCCD camera to reject photons that should not enter the detector. Details of the reciprocity principle were reported previously [19]. Finally, for a specific source $r_s$, detector $r_d$, and fluorophore position $r_f$, its sensitivity $G(r_s, r_d, r_f)$ is $G_{rr}(r_s - r_f) \times G_{od}(r_d - r_f)$. Some other factors such as fluorophore quantum yield should be considered for quantitatively estimating fluorophore concentration. These are disregarded here because this report mainly focuses on the regularization methods.

3. Results

3.1 Simulation setting

To evaluate the performance of the proposed method compared with those of methods mentioned above, preliminary simulations were implemented. We calculated the simulated fluorescence measurement $d$ for a predefined fluorophore object $m$ using Eq. 11.

$$d = Gm + n \tag{11}$$

where $n$ is 1% additive Gaussian random noise. Here 1% means that standard deviation of the random noise was set as 1% of amplitude of the simulated data, which approximates the random noise level of the EMCCD camera in our MEFT system.

Two patterns of fluorophore object were predefined:

1. 0–1 model: The fluorophore accumulates in a small local region with a uniform value of 1, while no fluorophore exists outside this region. This model is close to the condition of our phantom experiments in which the fluorophore is injected into a capillary. The model has a size of $1 \times 1 \times 1$ mm$^3$ and a central depth of 2 mm, and its central cross-section is shown in Fig. 4a.

2. Smooth model: The fluorophore also accumulates in the same local region as the 0–1 model; but smears into the adjacent voxels at a value of 0.5. Considering the phenomena of tumor spreading, we believe that this model is closer to the clinical condition. This model has a size of $2 \times 2 \times 1$ mm$^3$ and a central depth of 2 mm (Fig. 4b).

Optical properties of the volume surrounding the predefined fluorophore object were set at tissue-like values. Data of the 9×9 incident sources (spacing: 0.5 mm, scanning area: $4.5 \times 4.5$ mm$^2$) and the 15×15 detectors (spacing: 0.5 mm, observing area: $7.5 \times 7.5$ mm$^2$) were simulated. The total number of stimulated data was 18225. Reconstruction was performed up to a depth of 5 mm. The reconstructed whole medium with a volume of $5 \times 7.5 \times 7.5$ mm$^3$ was divided into $10 \times 15 \times 15 = 2250$

![Fig. 3](image)

**Fig. 3** Flowchart of the forward problem.
voxels. Each voxel is a cube 0.5 mm in length. For each pattern the simulation was repeated 5 times to evaluate the effect of random noise.

3.2 Simulation results

Figure 4b-e and Fig. 5b-e show the central cross-sections of the estimated fluorophore distribution for the 0–1 model and smooth model, respectively. To quantify reconstructing performance, four parameters (Tables 1 and 2) were computed: relative error (RE) of reconstruction to the predefined distribution (Eq. 12), and mean and deviation of the data in the region of the true value (ROT), which are used to evaluate whether a method accurately estimates the fluorophore concentration distribution in the ROT. Here, ROT refers to the region of predefined fluorophore object. Furthermore, to determine if serious artifacts exist or if data smear beyond the ROT, we computed the signal to background ratio (SBR), defined as the ratio of the mean in ROT to the mean outside ROT.

\[
RE = \frac{\|m_{\text{pred}} - m_{\text{true}}\|_2}{\|m_{\text{true}}\|_2}
\]

where \(m_{\text{pred}}\) is the reconstructed distribution and \(m_{\text{true}}\) is the predefined distribution.

In terms of RE, the two models were most accurately estimated by PWTR than by the other methods, as shown in Fig. 4c and Fig. 5c. In contrast, the cross-sections of Tikhonov regularization results (Fig. 4b, 5b) spread out into some voxels where the fluorophore was not predefined and resulted in comet-like distributions. This comet-like phenomenon was more significant when LSQR was used (Fig. 4d, 5d). Finally, sparsity regularization generated a bad estimation in the smooth model. As shown in Fig. 5d, the deepest row was obviously underestimated, while the maxima on this cross-section was 1.4, which was considerably larger than the actual value of 1. It seems that the values of voxels in the periphery are compressed into the center.

Although the mean in ROT was underestimated by all methods (Tables 1, 2), the proposed method yielded values closest to the true value. Furthermore, in terms of standard deviation (STD) in ROT, PWRT gave the lowest STD among all methods in the 0–1 model and a value very close to the true value in the smooth model. The closer values provided by PWTR reflect better preservation of the original fluorophore distribution. However, in the case of sparsity regularization, although the mean in ROT was close to the true value in the 0–1 model, data variability in the ROT was significantly greater than the true value, showing that the original distribution was impaired. SBR of PWTR and sparsity regularization were always higher than those of other methods, showing that the iterative weighted algorithms effectively suppressed the background data level. Finally, in terms of the effect of random noise, sparsity regularization was the most vulnerable to noise, while the results of other methods were not significantly affected by noise.

3.3 Experimental setting

We used an optical phantom consisting of a mixture of Intralipid, ink, and water to mimic the scattering and absorption properties of biological tissue. The optical properties of the phantom were measured by the double integrated spheres method [20]. At the excitation wavelength of 790 nm, the reduced scattering coefficient was 9.59 cm\(^{-1}\) and the absorption coefficient was 0.2 cm\(^{-1}\).

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Table 1 Performance of the methods used for reconstructing the 0–1 model (The best performance in each column is shown in red).

<table>
<thead>
<tr>
<th></th>
<th>RE</th>
<th>ROT mean</th>
<th>ROT std</th>
<th>SBR (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tikhonov</td>
<td>0.30±0.01</td>
<td>0.91±0.01</td>
<td>0.17±0.02</td>
<td>48.5±0.3</td>
</tr>
<tr>
<td>PWTR</td>
<td>0.12±0.02</td>
<td>0.95±0.01</td>
<td>0.10±0.02</td>
<td>63.2±1.4</td>
</tr>
<tr>
<td>sparsity</td>
<td>0.23±0.06</td>
<td>0.97±0.01</td>
<td>0.23±0.07</td>
<td>63.7±1.5</td>
</tr>
<tr>
<td>LSQR</td>
<td>0.42±0.00*</td>
<td>0.75±0.01</td>
<td>0.17±0.00*</td>
<td>40.0±0.2</td>
</tr>
<tr>
<td>true value</td>
<td>---</td>
<td>0</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* Actual value is lower than 0.005.

Table 2 Performance of the methods used for reconstructing the smooth model (The best performance in each column is shown in red).

<table>
<thead>
<tr>
<th></th>
<th>RE</th>
<th>ROT mean</th>
<th>ROT std</th>
<th>SBR (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tikhonov</td>
<td>0.31±0.00*</td>
<td>0.55±0.00*</td>
<td>0.25±0.01</td>
<td>38.1±0.5</td>
</tr>
<tr>
<td>PWTR</td>
<td>0.25±0.01</td>
<td>0.56±0.01</td>
<td>0.24±0.02</td>
<td>52.3±0.4</td>
</tr>
<tr>
<td>sparsity</td>
<td>0.50±0.04</td>
<td>0.54±0.01</td>
<td>0.44±0.02</td>
<td>52.3±0.6</td>
</tr>
<tr>
<td>LSQR</td>
<td>0.40±0.00*</td>
<td>0.52±0.00*</td>
<td>0.20±0.00*</td>
<td>32.1±0.1</td>
</tr>
<tr>
<td>true value</td>
<td>---</td>
<td>0.625</td>
<td>0.218</td>
<td>---</td>
</tr>
</tbody>
</table>

* Actual value is lower than 0.005.
Fig. 6 Optical phantom and fluorophore. For a clear view of the fluorophore, we did not allow the liquid phantom to immerse the capillary and injected ICG at a volume two times larger than that used in the actual experiment.

cm$^{-1}$. At the emission wavelength of 830 nm, the reduced scattering coefficient was 9.53 cm$^{-1}$ and the absorption coefficient was 0.3 cm$^{-1}$. These values are of the same order as the optical properties of human brain cortex [21] and female breast [22]. A fluorophore (indocyanine green, ICG; absorption peak wavelength 780 nm, emission peak wavelength 830 nm [23]; Keisei, Japan) was diluted with the background media (Intralipid and ink mixture) to a concentration of 10 μM. Two μL of liquid fluorophore was then injected into the center of a glass capillary tube by a pipetter. The tube (inner diameter 1.1 mm, outer diameter 1.5 mm) was fixed by a pair of sample holders in the tank (90 × 50 × 50 mm$^3$, Fig. 6). Both ends of tube were filled by clay to prevent background media from entering the tube. The liquid phantom was poured into the tank until the capillary was immersed. By adjusting the height of the phantom top surface, the bottom of the fluorophore capillary was set to be 2.0 mm lower than the phantom top surface. The CCD exposure time was set at 600 ms because the output power of incident light was only 0.2 mW. Fluorescent images of 10 × 10 incident positions, at intervals of 0.5 mm, were collected. We selected a 60 × 60 pixels ROI in each image and downsized them by a factor of 4. Therefore, 15 × 15 virtual detectors were generated and the total number of measurement data was 22500. Reconstruction was implemented for a space of 5 × 5 × 5 mm$^3$, which was divided into 8000 cubic voxels 0.25 mm in length. The experiment was repeated 3 times to evaluate the effect of system noise.

3.4 Experimental results

The reconstructed fluorophore distributions by Tikhonov regularization, PWTR, sparsity regularization and LSQR using fluorescence measurements of the phantom experiment are shown in Fig. 7. For reconstruction accuracy, we computed the volume above half the maximum (VHM), defined as the total volume of voxels with values larger than half of the maximum.

In the XZ cross-section, the reconstructed distribution by the proposed method (Fig. 7e) corresponded well to the actual fluorophore region, although a few artifacts existed at the right upper boundary. However, the distribution obtained by Tikhonov regularization (Fig. 7b) and LSQR (Fig. 7k) spread out smoothly in the entire space without clear boundary. In contrast, sparsity regularization (Fig. 7i) yielded an overly narrow distribution. By this method, the maximum value was also significantly larger than those of the other methods, showing the same “compressed” phenomenon as observed in the simulation results. In the Y direction, the proposed method did not provide a very satisfactory estimation (Fig. 7f). The distribution covered the actual fluorophore region, but a few voxels outside the region had relatively high values. As a result of the inaccuracy in the Y direction, VHM was also slightly underestimated as shown in the Fig. 8, although the PWTR still gave an estimation (2.5 ± 0.04 μL) closest to the actual fluorophore volume (2.0 μL) among all four methods.

4. Discussion

4.1 PWTR and Tikhonov regularization

The result obtained by Tikhonov regularization is characterized by a smooth distribution with unclear boundary. The estimated volume or cross-sectional area is thus always larger than the true value. Furthermore, the fluorophore concentration is usually underestimated. This is attributed to the smoothing effect of its norm 2 penalty term. This smooth and borderless regularization is used as the initial estimation in PWTR. Then, using the piecewise weight matrix, estimation converges to the actual situation with every iteration, i.e. values of voxels in the ROI approach the true value and boundaries become clearer. Using PWTR, the boundary and concentration of the fluorophore, i.e. those of disease-associated tissue, can be determined more easily.

4.2 PWTR and sparsity regularization

As mentioned before, PWTR inherits the iterative reweighted nature of sparsity regularization. The main difference between the two exists in the weight matrix. In sparsity regularization, weight of a specific element is defined as the absolute value of its estimated value in the last iteration. Therefore, those voxels with the largest value are assigned the greatest weight; i.e., they will be enhanced to the greatest extent in the next iteration. In contrast, other voxels with moderately large values are enhanced weakly; therefore, they are actually suppressed when compared with voxels with largest value. As a result, after several iterations, only large estimated values are retained in voxels with the largest initial value, while values of other voxels are reduced to zero. Although this method improves SBR, it results in poor image quality and underestimation of fluorophore distribution. Moreover, since the distribution of the fluorophore reporter is considered to represent tumor cells distribution, underestimation would mean that a part of the tumor may be missed, which would be clinically unacceptable.

On the other hand, in PWTR, a constant large weight is assigned to voxels of relatively large values. This allows
values of those voxels to increase to a similar extent at each iteration, while values of voxels treated as background are diminished. As shown in simulation and experimental results, this method produces locally limited fluorophore distributions and relatively high SBR. Furthermore, in the experiment, the fluorophore concentration in the capillary is uniform, and STD should be zero. The ROT STD for the result of PWTR is 1.1, while that of sparsity regularization is as large as 2.3. In addition, as shown in the Fig. 7e and Fig. 7f, the estimation by PWTR completely covers the ROT. These results prove that the local smoothness and completeness of the actual distribu-
4.3 Spatially variant regularization

In the proposed method, the weight for each voxel depends on its current reconstruction value. The weight matrix \( W \) thus spatially varies and the proposed method is a derivation of spatially variant regularization, which is a well discussed topic in the field of optical inverse problem. The objectives of spatially varying regularization can be divided into two categories:

1. To enhance the resolution and contrast of region far from the sources and detectors. For instance, Pogue et al. [24] used a radially varying regularization parameter for circular tomography, while Dehghani et al. [25] and Niu et al. [26] introduced sensitivity-dependent regularization for brain imaging.

2. To restrict the solution in several local regions. For example, Axelsson et al. [27] employed multi-spectral data to generate a spatially resolved weight matrix, and Shimokawa et al. [28] introduced a method of hierarchical Bayesian estimation in diffuse optical tomography to promote solution sparsity.

We focus on the second objective in this paper, but will attempt to integrate the first objective in the future. For the existing methods of the second category, we approve their positive effects in improving reconstruction accuracy, but notice that some methods require multi-spectral information [27] and measurement noise covariance matrix [28]. Particularly, for calculating the measurement noise covariance matrix, a large amount of measurements (20 times [28]) must be repeated under the same condition. This may prolong the measurement time considerably, thus restricting its potential in tracking some fast biomedical processes. Furthermore, due to the photo bleaching effect [29] by which a fluorophore will permanently lose the ability to fluoresce after certain light exposure, the detected fluorophore distribution may change during measurements that last a long time. Therefore, the calculation of noise covariance matrix will be inaccurate. Therefore, the feasibility of the hierarchical Bayesian estimation method in fluorescent tomography needs further evaluation.

4.4 Sources of inaccuracy

In the result of the proposed method, overestimation in the Y direction was observed. We attribute this to the inaccuracy of the initial estimation. Figure 9a shows an example of the initial estimation. In the central part, values in the Y direction are obviously larger than those in the Z direction. Because the same threshold was applied to all spatial directions to generate the weight matrix, considerably more voxels in the Y direction were thus considered as ROI voxels than those in the Z direction. Consequently, from the first iteration, more voxels in the Y direction were enhanced, leading to the imbalance in the final result. It is possible to solve the problem using a direction-dependent threshold, although the process of determining the threshold will become more complicated. Another solution is to apply additional knowledge as described by Axelsson et al. [27]. By utilizing additional knowledge to determine the first weight matrix, the influence of initial estimation can be avoided.

As shown in the Fig. 7e and Fig. 7f, some voxels at the boundary of the actual fluorophore region have values obviously inconsistent with those inside the region, although the fluorophore concentration should be uniform over the region. The inconsistency is partly attributed to our method of phantom construction. We utilized a glass capillary tube to make a fluorophore target that was restricted in a local region. However, the tube had a 0.2-mm thick glass wall. The glass wall is problematic due to its significantly different optical properties from those of the background. In the forward problem, we neglected this difference for simplicity. Therefore, it is possible that the reconstruction was negatively affected by the existence of the glass wall due to the mismatch between the forward model and the actual condition. In the future, replacement of the liquid phantom with a solid would obviate the use of the capillary tube and eliminate the effect of the glass wall.

4.5 Effect of \( \beta \)

In this study we introduced a piecewise weight matrix that involves an empirically determined threshold \( \beta \). The \( \beta \) is critical in balancing local smoothness and sparsity of solution. When \( \beta = 0 \), the PWTR degrades to Tikhonov regularization; and when \( \beta = 1 \), the PWTR approaches sparsity regularization. We evaluated the sensitivity of the reconstructed result to \( \beta \) by simulation. This simulation was conducted with the smooth model in the same conditions as described in the section 3.1 but with various \( \beta \). Figure 10 shows the dependence of relative errors of
The effect of Fig. 10

The initial estimation: Fig. 9

The estimated \( \beta \) by Eq. 7 and the corresponding relative error are shown by the red arrow.

4.6 Study limitations

As mentioned in the introduction, we studied a condition in which fluorophore is distributed only in a local region. However, in the clinical environment, it is possible that the fluorophore spreads out widely within the scanned area or that multiple fluorophore regions are present. For the former, it is possible to deliberately restrict the fluorophore to relatively local distribution by enlarging the size of the scanning area. For the latter, the proposed method is theoretically adequate to detect multiple fluorophore regions, although determination of the weight matrix becomes a more complicated problem, especially in the cases of multiple fluorophore regions with varying concentrations. Finding suitable solutions for these complexities is a challenge that we look forward to meeting.

Furthermore, tissue inhomogeneity is another critical factor impeding clinical application of FMT. Although only a homogenous optical phantom is addressed in this report, a test of the proposed method and the MEFT system in inhomogeneous environment will be conducted in the future. In this case anatomical information may be helpful, from which some groups are working on extracting local distribution of optical properties[30, 31]. We expect that these studies will facilitate preclinical and clinically applications of MFET.

5. Conclusion

In this report, we propose a novel piecewise weighted Tikhonov regularization method to solve the inverse problem of MEFT. Performance of the proposed method was evaluated by preliminary simulations and phantom experiments. Predefined fluorophore distributions were most accurately estimated by PWTR compared with the other general regularization methods, and PWTR yielded distributions with a clear boundary and local smoothness. Owing to the reflectance geometry, the technique of MEFT combined with the PWTR method has potential applications to early cancer diagnosis and intraoperative assistance. In the future, we shall challenge more complicated distribution of fluorophore reporters in more realistic environment.

References


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