Development of in-vivo fluorescence imaging with the Matrix-Free method

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Abstract. Non-contact Fluorescence Molecular Tomography is an emerging technique for imaging of fluorescent probes or proteins in live animals. One of the main characteristics of the non-contact acquisition systems in comparison to the usual fibre-based systems is the much denser boundary data sets that are created. When model-based reconstruction methods are used that rely on the inversion of a derivative operator, the large number of measurements poses a challenge since the explicit formulation and storage of the Jacobian matrix could be in general not feasible. In this paper we test a matrix-free method that addresses the problems of large data sets and reduces the computational cost and memory requirements for the reconstruction. More specifically we challenged the Matrix-Free method with in-vivo measurements from mice where fluorescence tubes of different but controlled concentrations are inserted, to assess the quantification performance of the method. We extended the test with simulations, using realistic geometries extracted from a mouse-atlas and including prior known information about the optical properties of tissue into the forward model.

1. Introduction

Recent advances in optical technologies and the development of several fluorescent proteins and switchable fluorochromes have evolved the field of optical imaging within the frame of Molecular Imaging, which is constantly gaining importance in Life Sciences. Compounds exhibiting fluorescent properties have recently played a major role in many biotechnology applications such as gene expression profiling, determining protein function as well as in analyzing cellular pathways and small-molecule/protein-protein interactions. Tomographic approaches have been utilized to image novel fluorescent agents with functional and molecular specificity through several millimeters to centimeters of tissue in vivo [1].

It is common for the developed fluorescence tomographic systems [2, 4, 3] to employ non contact detection schemes in order to retrieve the fluorescence measurements by means of an optical system that projects the surface of the medium onto a CCD camera producing a large amount of measurements from the pixels of the CCD sensor. Provided that the signal-to-noise ratio is good enough, a larger dataset can provide an improved solution to the inverse problem; firstly, by the increased spatial resolution of the reconstruction [5] and secondly, by reducing the illposedness of the problem by the higher information content [6].
In order to solve the inverse tomographic problem with a large number of data the requirements on the computational hardware become immense. In the most common reconstruction schemes the system Jacobian is built and stored in the computer memory and the size of the Jacobian matrix depends on the number of measurements acquired and the resolution of the geometry used for the numerical solution. Practically this imposes a limit for the maximum size of the Jacobian depending on the amount of computer memory that is available.

The problem of large datasets has been investigated for the diffuse optical tomography case using an approach based on the analytical solution to the diffusion equation for geometries where the solution is available in [8] and [7]. They report on the ability to reconstruct absorption heterogeneities using a number of source-detector pairs in the order of $10^8$.

Similarly, in [9] a hybrid Fourier encoding for the measurements has been used to successfully reduce the effect of the large datasets for certain geometries. In addition, compression with wavelets has been implemented for reconstructions from simulations in [10] on realistic geometries.

Our approach initially presented in [11], is based on a formulation of the diffusion approximation for the fluorescence case and uses a matrix free formulation. Hence, the explicit calculation and storage of the Jacobian is avoided by replacing it by a vector times matrix operator and a vector times adjoint matrix operator. Also, the matrix free formulation has the property of requiring equal computer power independent on the number of detectors used, thus making it ideal for imaging with large number of measurements.

In this paper we initially present in-vivo reconstructions from mouse experiments with controlled concentrations of Alexa Fluor 647 (Invitrogen, France). For the reconstructions we have used a slab approximation for the mouse geometry and the matrix-free method. Having five different sets of measurements with different concentrations of the fluorochrome, results are also presented on the quantification capabilities of the method.

Since fluorescence tomography is a functional imaging modality in the recent years the inclusion of prior geometric information in the inversion of fluorescence is becoming a new common practice. Anatomical prior information of the biological tissue is usually acquired from a second modality on site, like X-ray Computed Tomography (XCT) [16, 15] or Magnetic Resonance Imaging (MRI) [14] to increase imaging resolution. A feasibility study using 2D simulations has shown the importance of a-priori structural and functional information (optical parameters) for the reconstruction of fluorescence targets in the brain [23]. To this end, in addition to the in-vivo experiments we performed a simulation study, where anatomical information from a mouse atlas was incorporated in the forward problem and a fast matrix-free solution of the inverse problem is presented.

This paper is organized as follows. In subsection 2.1 we describe the problem using a set of two paired diffusion equations for the excitation and the fluorescence wavelength, and construct the derivative operators. Subsection 2.2 defines the reconstruction as an optimization problem and gives an insight to the scalings that were introduced to improve the convergence of the problem. Subsection 2.3 presents the matrix-free approach. In vivo reconstructions from mice and the quantification properties of the method is the subject of section 3, and finally reconstructions using simulations with realistic geometries and a-priori known optical parameters are presented in section 4.
2. Matrix Free Method

2.1. Forward Model

The forward model for the continuous-wave fluorescence tomography problem is given by the coupled diffusion equations at the excitation and emission wavelengths \( \lambda_e \) and \( \lambda_f \), respectively:

\[
(-\nabla D(r, \lambda_e) \nabla + \mu_a(r, \lambda_e)) U^{(e)}(r) = q(r) \\
(-\nabla D(r, \lambda_f) \nabla + \mu_a(r, \lambda_f)) U^{(f)}(r) = U^{(e)}(r) h(r, \lambda_f),
\]

with Robin boundary condition at both wavelengths, \([12]\),

\[
U(\xi) + 2 \zeta D(\xi, \lambda) \frac{\partial U^{(e/f)}(\xi)}{\partial n} = 0, \quad \xi \in \partial \Omega
\]

where \( D \) and \( \mu_a \) are the wavelength-dependent diffusion and absorption coefficients, \( q \) is a boundary source at the excitation wavelength, \( \zeta \) is a boundary term incorporating the refractive index mismatch at the surface of the medium, \( n \) is the outward surface normal at \( \xi \), and \( U^{(e)} \) and \( U^{(f)} \) are the photon density fields at the excitation and emission wavelength, respectively.

We have defined the wavelength-dependent \textit{fluorescence yield coefficient} \( h \) for the domain \( \Omega \):

\[
h(r, \lambda) = \eta(\lambda)c(r), \quad r \in \Omega
\]

where \( \eta(\lambda) = \epsilon \gamma(\lambda) \) is the product of the quantum yield \( \gamma(\lambda) \) and the extinction coefficient \( \epsilon \) for the fluorochrome at wavelength \( \lambda \), and \( c(r) \) is the concentration distribution of the fluorochrome. The contribution of \( h \) to the absorption at \( \lambda_e \) is here considered negligible.

The exitance at boundary \( \partial \Omega \) for both wavelengths is given by the boundary operator

\[
y(\xi) = -D(\xi, \lambda) \frac{\partial U(\xi)}{\partial n} = \frac{1}{2\zeta} U(\xi), \quad \xi \in \partial \Omega
\]

The exitance distribution on the surface is sampled with an array of detectors (e.g. a CCD camera), providing a discrete set of measurements \( g_d^{(e)} \) and \( g_d^{(f)} \) at both wavelengths, such that

\[
g_d^{(e/f)} = \mathcal{M} \left[ y^{(e/f)} \right] := \int_{\partial \Omega} y^{(e/f)}(\xi) w_d(\xi) d\xi
\]

where \( w_d \) is the sensitivity profile of detector \( d \) on the boundary.

Assuming that the optical parameters \( D(r, \lambda) \) and \( \mu_a(r, \lambda) \) are known, the reconstruction problem consists in finding the fluorochrome concentration \( c(r) \). First consider the problem of reconstructing \( h \). Eq. (1-5) define the forward problem which maps distribution \( h(r, \lambda_f) \) to fluorescence data \( g_d^{(f)} \):

\[
g^{(f)} = F(h)
\]

To solve Eq. (1) and (2), we use a finite element model. Parameters \( D, \mu_a \) and \( h \) are expressed as finite-dimensional vectors \( D, \mu_a, h \in \mathbb{R}^P \) whose elements are the coefficients of a basis expansion

\[
D(r) \approx \sum_{k=1}^P D_k b_k(r), \quad \mu_a(r) \approx \sum_{k=1}^P \mu_k b_k(r), \quad h(r) \approx \sum_{k=1}^P h_k b_k(r)
\]

with basis functions \( \mathcal{B} = \{b_k(r), k = 1 \ldots P\} \). The fields \( U \) are expanded in the basis of a finite element methods (FEM):

\[
U(r) \approx \sum_{\ell=1}^N U_{\ell} v_{\ell}(r)
\]
hence the diffusion equation can be written as a linear system

\[ K(D, \mu)U = Q \]  

(9)

where \( K \in \mathbb{R}^{N \times N} \) is a system matrix assembled from element contributions that depend on the parameter distributions, and \( U \) is the vector of basis coefficients representing the photon density distribution in basis expansion \( V = \{ v_\ell(r), \ell = 1 \ldots N \} \). The forward model in the discrete setting consists of solving

\[ K_{\lambda_f} U^{(e)} = Q, \]

(10)

\[ K_{\lambda_f} U^{(f)} = h \odot U^{(e)}, \]  

(11)

with \( K_{\lambda_f} = K[D(\lambda_f), \mu_a(\lambda_f)] \), \( K_{\lambda_e} = K[D(\lambda_e), \mu_a(\lambda_e)] \) and \( \odot \) representing element-wise multiplication. Together with the boundary operator, the forward model can now be expressed in the linear form

\[ g^{(f)}_Q = F_Q(h) = A_Q h := M \left[ K_{\lambda_f}^{-1} h K_{\lambda_e}^{-1} Q \right] \]  

(12)

where \( A_Q(h) \) is the discrete matrix representation of the forward operator. Examination of the expression in Eq. (12) indicates that the Jacobian can be constructed using the adjoint formulation

\[ J^{(h)}(sd, k) = U^{(e)} s, k U^{(f)} f, d + \]  

(13)

where \((sd)\) denotes a row index constructed from an ordering of source index \( s \) and measurement index \( d \), and

\[ K_{\lambda_e} U^{(e)} = Q_s, \]  

(14)

\[ K_{\lambda_f} U^{(f)} = Q_d, \]  

(15)

are the solutions of the direct and adjoint problems.

2.2. Inverse problem

In general we pose the inverse problem as an optimisation problem

\[ \hat{x} = \arg \min_x \left[ \Phi(x) := \frac{1}{2} \| g^{(f)}_{\text{meas}} - F(x) \|^2 + \alpha \Psi(x) \right] \]  

(16)

with regularisation term \( \Psi \) and hyperparameter \( \alpha \). Since \( F(x) = Ax \) is linear, an iterative solution to Eq. (16) is obtained for example by the damped Gauss-Newton scheme:

\[
\begin{align*}
\left( A^T A + \alpha \Psi''(x^{(k)}) \right) x^{\delta} &= A^T (g^{(f)}_{\text{meas}} - Ax^{(k)}) - \alpha \Psi'(x^{(k)}) \\
\tau_k &= \arg \min_\tau \Phi(x^{(k)} + \tau x^{(k)}) \\
x^{(k+1)} &= x^{(k)} + \tau_k x^{\delta}
\end{align*}
\]  

(17)

where \( \tau \) denotes the step length. If the regularisation is quadratic, \( \Psi(x) = \frac{1}{2} \| Lx \|^2 \), we have the direct reconstruction formula

\[
A^T A + \alpha L^T L \hat{x} = A^T g^{(f)}_{\text{meas}} \]  

(18)

where \( L \) is for example the Cholesky factorisation of a quadratic Markov Random field. In the results presented in this paper, we consider only the simplest zero-order Tikhonov regularisation \( L = I \).
Due to uncertainties in laser power, detector gain, and losses we cannot expect the forward model to be compatible with the measured data. To avoid problems due to the model mismatch, we employ data normalization by making use of the available excitation data, and thus we do not require absolute measurements. The optimization problem Eq. (16) is transformed into the rescaled problem

\[
\hat{x} = \arg\min_x \frac{1}{2} ||\tilde{g}^{(f)}(x) - \tilde{F}(x)||^2 + \alpha \Psi(x) \tag{19}
\]

We used the Normalized Born approximation [20], which is extensively used in fluorescence tomography.

\[
g^{(f)}_{\text{meas}} \rightarrow \tilde{g}^{(f)} = g^{(f)}_{\text{meas}}, \quad F(x) \rightarrow \tilde{F}(x) = \frac{g^{(e)}_{\text{meas}}}{g^{(e)}_{\text{proj}}} F(x) \tag{20}
\]

Where \( g^{(e)}_{\text{proj}} \) denotes the calculated data in the excitation wavelength.

2.3. Implementation of the matrix-free method

When faced with solving the linear problem Eq. (19), the explicit computation and storage of the matrices \( A^TA \) and \( A^Tg^{(f)}_{\text{meas}} \) is costly and often intractable for large scale problems. This is mainly due to the large size of the Jacobian matrix \( A \). As an example, a problem with 30 sources and 475 detectors solved on a geometry with 7812 elements would need about 890MB of storage for each wavelength. The problem is more intense when more samples from the CCD image are required or a high resolution mesh is used to represent the domain in the solver. When using a Krylov solver for the linear problem we require to construct the set of basis vectors

\[
\{ z, Hz, \ldots H^jz \} \tag{21}
\]

where \( z = A^Tg^{(f)}_{\text{meas}} \) and \( H = A^TA + \alpha L^TL \). In our approach therefore, we represent the forward and adjoint multiplication by the Jacobian implicitly, using a function that returns the result of the operation. To be specific, we use the Matlab ‘gmres’ function to solve Eq. (19), and pass it a function that calculates the forward and adjoint solutions for any intermediate vector generated in the solution iteration. The method is summarised in Algorithm 1.

**Algorithm 1** Schematic of reconstruction method

For each source \( s \), calculate forward excitation fields \( U^{(e)}_s \)
Calculate \( z = A^Tg^{(f)}_{\text{meas}} \) using adjoint solver
Inside GMRES solver :
\( v_0 = z \).
for all Krylov basis vectors \( v_j, j = 1 \ldots \) do
  Update fluorophore concentration \( h = \sum_i \eta_i(\lambda_f) v_{j-1,i} \)
  Calculate forward projection \( r = Ah \)
  1 Solve for every source \( s \) : \( K_{\lambda_f} U^{(\lambda_f)}_s = h \odot U^{(e)}_s \)
  2 Calculate : \( r^{(f)}_s = M[U^{(\lambda_f)}_s] \)
  Adjoint Calculation \( x^{(f)} = A^T \cdot r^{(f)}_s \)
  1 Solve for every source \( s \) : \( K_{\lambda_f} V = r^{(f)}_s \)
  2 \( f = f + V \odot U^{(e)}_s \)
end for
3. In-vivo quantification experiments

In this section we assess the quantification properties of the matrix-free method using in-vivo measurements on mice. More specifically we used a balb/c mouse where capillary of Alexa Flou 647 of controlled concentrations 0.9µM (micromoles/litre), to 60.5µM was inserted hypodermically in the ventral side of the mouse. The acquisition was performed using the experimental setup in FORTH, [21, 22]. The system incorporates a solid state laser at 635nm (Coherent, USA). The laser light is directed inside a light shielded imaging chamber and enters a laser scanning device (Scancube 7, Scanlab, Germany) which consists of two mirrors driven by miniaturized galvanometer motors. The angular movement of the motors is controlled by a custom made computer software for directing the laser beam in a controlled manner to the sample with the aid of a large rectangular mirror (first surface mirrors, 4-6 Wave, 73 mm 116 mm, Edmund Optics). The sample is placed on a transparent glass plate with an anti-reflection coating (Glassplate, High AR coated 96-99% (400-700nm)) and receives the illumination from below. Images are captured by a thermoelectrically cooled 16bit CCD camera with a chip size of 1024x1024 pixels (Andor Corp., DV434, Belfast, Northern Ireland), which is mounted on the upper plate of the imaging box, on the other side of the illumination. The CCD camera is equipped with a SIGMA 50mm f/2.8 objective (Sigma Corporation, Tokyo, Japan) which focuses on the sample’s surface. A filter wheel mounted in front of the CCD camera lens enables the capturing of images at 2 wavelength positions. The first being at the emission range of the fluorophore, for taking the Fluorescence set of images and the second is at the excitation wavelength taking the Intrinsic set of data.

Transmission measurements were acquired with the laser on the ventral side of the mouse and the camera facing the dorsal side, figure 1. The mouse was gently compressed by a transparent glass plate of 1mm thickness conforming the subject into a slab geometry. The measurements consisted of 36 sources position each collecting two 512x512 images, one for each wavelength. For the reconstructions, a slab-like geometry of dimensions 41mm x 41mm x 12mm was used with 5733 nodes and 4800 voxel-elements. For the optical parameters we assumed \( \mu_a = 0.06mm^{-1} \) and \( \mu_s' = 1.6mm^{-1} \) for the 615nm excitation and \( \mu_a = 0.05mm^{-1} \) and \( \mu_s' = 1.5mm^{-1} \) for the 700nm emission wavelengths. We set 1458 detectors positions by sampling the images, which for the 36 sources created two vectors of 48285 measurements each.

The results for the five different experiments are presented in figure 2, as slices that go through the center of the reconstructed capillaries in each case and represent a coronal plane slice along the y-axis (top) and a a transverse plane slice along the z-axis (bottom). The same results are also presented as iso-surfaces in 3D in figure 3, where the white light image of the mouse is

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Figure 1. (left) The whitelight image of the mouse used for the Alexa Fluor capillary inserted in the ventral side of the mouse. (right) The spectral response for the Alexa Fluor 647 where we show for reference the laser line at 635nm.
Figure 2. Reconstructions from an in-vivo mouse where capillaries filed with Alexa Fluor 650 of controlled concentrations have been inserted in the ventral side of the mouse, while the camera "looks" on the dorsal side. The different reconstructions correspond to concentrations a)60.5 \( \mu M \), b)22 \( \mu M \), c)8.5 \( \mu M \), d)2.2 \( \mu M \) and e)0.9 \( \mu M \). The slices go through the center of the reconstructed capillaries and represent a coronal plane slice along the y-axis (top) and a transverse plane slice along the z-axis (bottom).

rendered in the background as a reference. The average time for the reconstruction was 50sec, in a Pentium 2Ghz machine with 2GB of memory.

Since, we had experiments with different concentrations of Alexa Fluor, we decided to assess the quantification accuracy of the matrix-free method. More specifically, we calculated a histogram of the reconstructed values for each experiment and took the mean value of the voxels with the highest concentrations. The results are presented in the figure 4, versus the experimentally known concentrations. The curve that connects the points on the graph, is almost linear, up to some experimental error, and shows the correspondence of the real concentrations to the reconstructed values.

4. Simulations with realistic geometries and prior known optical parameters
In the latest approaches in Fluorescence Imaging we see the inclusion of structural information for the mouse anatomy, either through the use of a second modality on-site, (XCT,MRI) or through the utilization of a mouse atlas, we decided to test the ability and performance of the Matrix-Free method on the use of realistic geometries. As an initial approach to the feasibility of the method with realistic geometries we decided to use an already existing atlas for the creation of simulated measurements and the reconstruction for a fluorescence target. More specifically we used geometries extracted from Digimouse, [17, 18], an adult mouse atlas generated using...
Figure 3. Reconstructions from an in-vivo mouse where capillaries filed with Alexa Fluor 650 of controlled concentrations. 3D renderings of the reconstructed capillaries, for the different concentrations a) 60.53 $\mu$M, b) 22 $\mu$M, c) 8.54 $\mu$M, d) 2.23 $\mu$M and e) 0.91 $\mu$M. The white light image of the mouse from the camera is rendered as a reference. The average time for the reconstruction was 50sec.

We coregistered CT and cryosection images of a 28g nude normal male mouse. We extracted the surfaces of the head, brain and skull, from the atlas and using iso2mesh [19] mesh creation algorithm we created a mesh of 11714 nodes, and 60822 elements. An outline of the mesh with the three distinct regions can be seen in figure 5.

For the forward model the values for $\mu_a$ and $\mu'_s$ for the brain and the rest of the tissue were taken from [13] for the wavelengths of excitation at 682nm and emission at 710nm as seen in table 1. The fluorescence target had the shape of a rod and was placed inside the region of the brain as seen in figure 6 on the left side. We assumed a fluorescence response of 1.7 for the rod and zero for the rest of the tissue. 20 sources were placed in the ventral side of the mouse and 1584 detectors positions were assumed on the dorsal side. In total that produced 31680 measurements in each wavelength. Simulated data were created using the forward solution on the above setup. We added 5% multiplicative gaussian noise to the data and used them for the
Figure 4. Plot of the reconstructed concentration for Alexa Fluor 647 from five experiments with different capillaries versus the experimentally known concentration.

Figure 5. The geometrical model extracted from an atlas for the mouse head. The different regions correspond to different absorption and scattering parameters for the forward model.

Table 1. Optical parameters for the 682nm and the 710nm that were used for the simulation of the three regions in the mouse head.

| Wavelength | Brain | Skull | Other tissue |
|------------|-------|-------|--------------|
| 682nm      | $\mu_a = 0.023$ | $\mu_s' = 1.99$ | $\mu_a = 0.05$ | $\mu_s' = 1.29$ |
| 710nm      | $\mu_a = 0.017$ | $\mu_s' = 1.86$ | $\mu_a = 0.03$ | $\mu_s' = 1.2$ |

reconstruction with a Matrix-Free method. The reconstruction took 54sec. on a 2GHz Pentium with 2GB memory machine. The resulting reconstruction can be seen in the figure 6 on the right side, while slices along the center of the target and the reconstructed target can be found in figure 7.

5. Conclusions
In [11] we introduced a matrix-free method for multiwavelength reconstructions for Fluorescence Tomography that is able to deal with the large amount of data that are created by the non-


contact acquisition and produce fast and good resolution reconstructions. Following that work, and having in-vivo applications in mind we tested the Matrix-Free method with in-vivo data from a mouse where fluorescence targets of different concentrations were inserted. Using the Matrix-free method we managed to reconstruct for the positions of the fluorescent tubes and a relative quantification accuracy and sensitivity. More specifically in figure 4, the algorithm demonstrated an almost linear response between the controlled concentrations and the reconstructed ones.

Apart from the slab-like reconstruction used for the in-vivo experiment and having in mind imaging applications in the brain, we also tested the algorithm with the use of realistic geometries, created from a mouse atlas. Anatomical and prior known optical properties were implemented in the forward model and demonstrated a reconstruction, figure 6 and figure 7, from simulated data which took 54sec to converge.

As future work we propose the use of a second modality like XCT of MRI to acquire a case specific mesh, and in the further extent the use of generic atlases to adopt a mesh to the specimens under investigation.
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