A High Precision Method for Analysis of Fluorescence Lifetime Imaging Microscopy Data Based on Levenberg-Marquardt Technique

Liu Yang Zhou Yan Liu Yuliang

Optoelectronic System Laboratory, Institute of Semiconductors, Chinese Academy of Sciences, Beijing 100083, *China*

Abstract A high precision Levenberg-Marquardt (LM) iterative algorithm for the analysis of fluorescence lifetime imaging microscopy (FLIM) data is reported. The performance of this method was tested on the time-resolved fluorescence intensity images of fluorescence lifetime standard dyes and real biological images. The method is applicable for various fluorescence decay functions and has better estimation precision based on iterative nonlinear least squares minimization algorithm. It indicates that the LM iterative technique for FLIM analysis represents a precise and versatile method that enables practical applications of FLIM in biology, biochemistry, biophysics, and medical diagnosis.

Key words imaging systems; Levenberg-Marquardt; nonlinear least squares optimization; time-gated fluorescence lifetime imaging microscopy; fluorescence lifetime map

OCIS codes 110.2960; 100.2960; 110.1758

一种基于列文伯格-马克夸特的高精度荧光寿命计算成像方法

刘 洋 周 燕 刘育梁

中国科学院半导体研究所光电系统实验室, 北京 100083

摘要 介绍了一种用于荧光寿命图像数据分析的高精度列文伯格-马克夸特(LM)迭代算法。该算法的性能经过标准荧光寿命试剂以及生物图像的算法验证。该算法适用于不同的荧光衰减模型,相对于普通的非线性最小二乘估计方法具有更高的精度。结果表明,列文伯格-马克夸特算法是一种高精度、适用性广的荧光寿命图像计算方法,可以满足生物学、生物化学、生物物理学、医学诊断等实际应用的需求。

关键词 成像系统; 列文伯格-马克夸特; 非线性最小二乘优化方法; 门控荧光寿命成像系统; 荧光寿命图像

中图分类号 O433 **文献标识码** A

doi: 10.3788/LOP52.101102

1 Introduction

Fluorescence lifetime imaging is a nondestructive optical method extensively used in biology, biomedical and biochemical applications. Fluorescence lifetime map could quantitatively measure the intracellular microenvironment such as pH, Ca^{2+} concentration, oxygen concentration, since lifetime reveals the non-radiative decay phenomena in the cellular environments which are hardly quantified via steady-state intensity imaging[1], and it could monitor fluorescence resonance energy transfer in which intensity contrast is limited by the spectral overlap[2].

Fluorescence lifetime imaging microscopy (FLIM) is mainly performed either in the frequency or time domain. In the frequency domain, fluorescence emission is excited by sine modulated light, in which lifetime is computed through a phase shift and an amplitude modulation in the emission intensity. In the time domain,

收稿日期: 2015-03-13;**收到修改稿日期**: 2015-04-29; **网络出版日期**: 2015-09-23

作者简介: 刘 洋(1988—),男,硕士研究生,主要从事生物医学图像处理方面的研究。E-mail: liuyang0625@semi.ac.cn

导师简介: 刘育梁(1966—),男,研究员,博士生导师,主要从事光电图像处理、光纤传感等方面的研究。

E-mail: ylliu@semi.ac.cn

fluorescence sample is excited by short pulsed light, and fluorescence is recorded as intensity decay curves. The method presented in this paper is focused on time-domain FLIM data[3].

In the context of time-domain FLIM, the measured fluorescence response (fIRF) from the fluorescence sample by the short pulse laser is the convolution of intrinsic fluorescence impulse response function (iIRF) with instrument response function (IRF)[4]. To nearly resemble the iIRF, it always uses picosecond or femtosecond laser pulse to resemble the function excitation which can almost eliminate the influence of convolution. The influence of the IRF by short picosecond gate or high speed detector is also ignored. For the analytic result, the decay curve of iIRF is approximately the same as the parameters of fIRF, which can compute the precise value of fluorescence lifetime[5].

The most commonly used analysis technique for time-resolved FLIM data is rapid lifetime determination (RLD) and linear least squares (LLS) method. These methods both model the iIRF as a single exponential function and compute the lifetime value by linear least squares method or equation solution directly[6]. There are, however, some limitations in these methods. First, the exponential model parameters are actually nonlinear and the exponential mode linearization abandons some estimation accuracy. Second, a single-exponential model can not represent most iIRF of fluorescence samples, as multi-exponential expansion is a much more general model form considering complex biological characterization.

A nonlinear least squares optimization for FLIM data analysis based on Levenberg-Marquardt (LM) iterative technique is firstly introduced and validated. In this method, the lifetime value at each pixel of FLIM image is optimized based on LM iterative condition. Since the parameters of iIRF are nonlinear, it is more precise to apply nonlinear least squares optimization. For the time domain FLIM image computation, the LM algorithm combines the advantages of the steepest gradient descent method with the Newton method. The algorithm obtained its operating stability from the steepest gradient descent method, and adopted the accelerated convergence in the minimum vicinity from the Newton method[7-8]. Not limited by the iIRF models, LM algorithm can estimate the parameters of multi-exponential or other expansion forms that facilitate various FLIM applications.

The performance of this algorithm is validated by fluorescence lifetime standard and time-resolved biological image data. The lifetime images are compared with the results processed by RLD and LLS methods, and we compute the mean lifetime value and its histogram of region of interest (ROI). The LM algorithm has better estimation precision, smaller standard deviation and more centralized numerical distribution. This method will facilitate applications of FLIM requiring really high estimation precision, such as FLIM imaging for cancer diagnosis.

2 Methods

2.1 Least squares optimization method of fluorescence lifetime parameter

In the context of time-domain FLIM, the objective of data analysis is to obtain the lifetime parameter of iIRF using the series of time-gated fluorescence intensity images $I(r,t_n)$, here r denotes the pixel location and t_n is the delay time. The iIRF model of fluorescence sample to estimate could be defined as $I(t)=\sum_{i=1}^{k}a_i\exp(-t/\tau_i)$. Here, a_i denotes the i^{th} exponential coefficient and τ_i denotes the i^{th} lifetime value. The problem that estimates the parameters of this exponential model can be framed as nonlinear least squares optimization.

The cost function at the r^{th} pixel is defined as the sum of squared residuals of the estimated decay:

$$S(\boldsymbol{\beta};t)=\frac{1}{2}\sum_{j=1}^{n}\left[I_j(\boldsymbol{\beta};t)-y_j\right]^2=\frac{1}{2}\sum_{j=1}^{n}f_j(x)^2\equiv\frac{1}{2}\boldsymbol{F}(x)^{\mathrm{T}}\boldsymbol{F}(x), \tag{1}$$

where $\boldsymbol{F}(x)$ is the vector-valued function consists of $f_j(x)$. The computation of lifetime τ is to find the vector

$\boldsymbol{\beta}$ that minimizes the cost function $S(\boldsymbol{\beta};t)$ and consists of a_i, τ_i based on the exponential iIRF. Since the residuals are not linear to the $S(\boldsymbol{\beta};t)$, it is necessary to use a precise and fast convergence updating method.

2.2 Levenberg-Marquardt iterative lifetime computation method

Like other nonlinear optimization method, LM is an iterative numerical minimization algorithm which gradually converges to a minimum with an initial guess. To approximate the final estimated parameter vector, the parameter vector β is replaced by a new vector $\beta+\delta$ in each iterative step. To update δ, the function $I(\beta;t)$ is expanded by the first-order Taylor approximation[9].

$$I(\beta+\delta;t)\approx I(\beta;t)+\boldsymbol{J}\delta, \tag{2}$$

where the Jacobian matrix $\boldsymbol{J}=\partial I(\beta;t)/\partial\beta$ is the gradient of I respect to β. Based on this, the cost function could be represented in vector notation,

$$S(\beta+\delta;t)\approx\left\|y-I(\beta;t)-\boldsymbol{J}\delta\right\|^2. \tag{3}$$

Since the gradient of the cost function $S(\boldsymbol{\beta};t)$ is zero at the minimum, it gives the δ and the final estimated lifetime parameter by taking the deviation with respect to δ,

$$\left(\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J}\right)\delta=\boldsymbol{J}^{\mathrm{T}}\left[y-I(\beta,t)\right]. \tag{4}$$

This is the iterative condition of Gauss-Newton method which converges quickly near the minimum. However, the method takes large, uncontrolled steps and will fail to converge if the initial guess is bad. To remedy this shortcoming, Levenberg and Marquardt suggested damping the $\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J}$ matrix by a diagonal cutoff. The LM algorithm updates the δ according to

$$\left[\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J}+\lambda\ \mathrm{diag}(\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J})\right]\delta=\boldsymbol{J}^{\mathrm{T}}\left[y-I(\beta,t)\right], \tag{5}$$

where $\boldsymbol{J}^{\mathrm{T}}\boldsymbol{J}$ is a positive-definite, diagonal matrix representing the relative scaling of the parameters and λ is a damping parameter adjusted by the algorithm. When λ is large, the method takes a small step in the gradient direction[9]. As the method is near a solution, λ is chosen to be small and the method converges quickly via the Gauss-Newton method.

For the time-resolved FLIM image data, the implementation of LM algorithm consists of four steps iteratively, the algorithm is applied pixel by pixel.

1) Update the function and Jacobian matrix values based on the current parameters (given the initial guess), update the scaling matrix and damping parameter λ;

2) Compute a new parameter step vector and evaluate the function based on the new parameter value;

3) Accept or reject the parameter step δ depending on whether the cost function has decreased at the new parameter β;

4) Stop if the algorithm has met any of the desired convergence criteria or has exceeded the limit of iterations or function evaluation condition.

2.3 Method validation on FLIM data

The performance of the introduced algorithm was tested on: 1) time-resolved fluorescence lifetime standard intensity images; 2) time-resolved nematode fluorescence intensity images.

2.3.1 Instrument for FLIM measurement

The FLIM imaging was accomplished using a time-domain wide-field time-gated instrument. The key component of the FLIM system was an ICCD detector (PI-MAX4, Princeton Instrument) coupled to a 70 mm fixed focus lens. A series of pulsed laser (372 nm, 70 ps FWHM, 1 MHz) was generated by a picosecond laser diode (PDL 800-B, PicoQuant GmbH), which was used for excitation. A dichroic mirror was used to separate the excitation pulse and the fluorescence emission[10]. The current system could acquire the time-resolved images with a delay resolution of 10 ps.

2.3.2 Fluorescence lifetime standards and nematode images

To validate the performance of LM algorithm, a series of time-resolved fluorescence intensity images with known lifetime were acquired. We recorded FLIM images of two different fluorophores separately. The fluorophores are rhodamin B (Acros) and 9-cyanoanthracene (TCI) dissolved in ethanol. The solution in the quartz cuvette was placed on the sample stage.

Another sample of nematode containing green fluorescent protein (GFP)[11] offered by Becker & Hickl GmbH company was validated by LM algorithm. A series of time-gated images were acquired within time windows of 0.5 ns starting from the excitation pulse.

3 Results and discussion

3.1 Validation on fluorescence lifetime standards

The lifetime maps of rhodamin B and 9-cyanoanthracene solvent processed by LM algorithm are shown in Fig. 1. The lifetime values (mean lifetime ± standard deviation) of them are (2.45±0.11) ns (rhodamin B) and (10.24±0.88) ns (9-cyanoanthracene), which are computed from the region of interest (the region that laser illuminates for these images). These values are in good agreement with the same fluorescent standards as others reported[12]. The histograms of two FLIM maps shown in Fig. 2, illustrate that this algorithm is accurate and has uniform distribution.

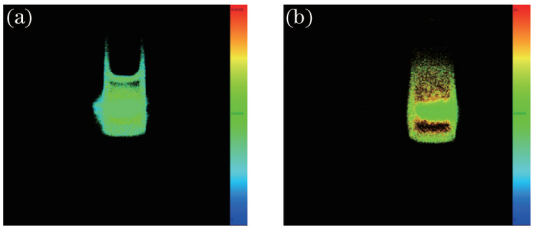

Fig.1 Validation results of the algorithm on FLIM images of standard fluorophores.
(a) Lifetime map of rhodamin B solvent; (b) lifetime map of 9-cyanoanthracene solvent

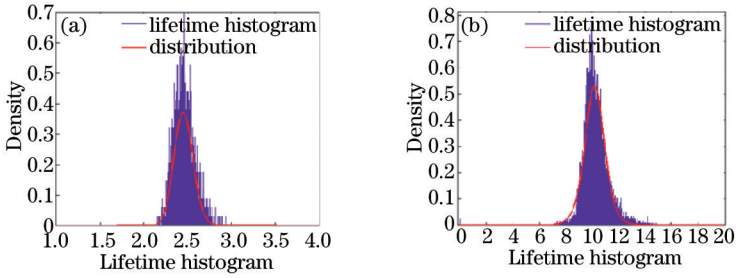

Fig.2 Histograms of lifetime maps. (a) Rhodamin B solvent; (b) 9-cyanoanthracene solvent

3.2 Validation on nematode images

Results from the analysis of FLIM images of nematode containing GFP are given in Fig. 2. Besides the LM algorithm [Fig. 3(c)], the images processed by RLD [Fig. 3(a)] and LLS [Fig. 3(b)] are shown as well. The lifetime values of the three methods are (3.98±1.23) ns (RLD), (3.76±1.14) ns (LLS), (3.91±1.07) ns (LM), respectively. The histograms of the three methods are illustrated in Fig. 4, it is obvious that the LM algorithm has better precision, including smaller standard deviation quantitatively and more centralized lifetime distribution qualitatively.

4 Conclusion

To summarize, we have presented a new LM iterative method for FLIM data analysis. The method has been validated on the FLIM data from fluorescence lifetime standards, time-resolved images. The main advantage of the introduced method is smaller standard deviation and applicability to different iIRF models. Current effort about this work focuses on LM algorithm combining with optimization of the number and the delay

interval of time-resolved fluorescence intensity images to enhance the FLIM image quality.

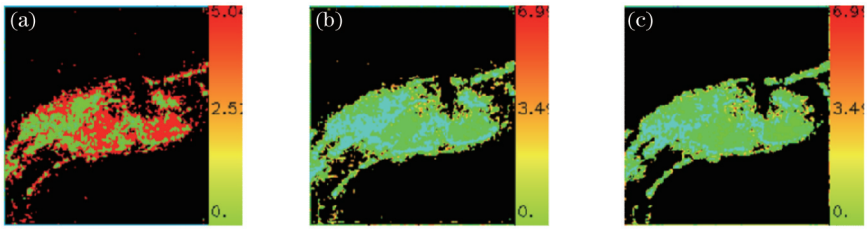

Fig.3 Lifetime images processed by (a) rapid lifetime determination; (b) linear least squares method; (c) LM iterative algorithm

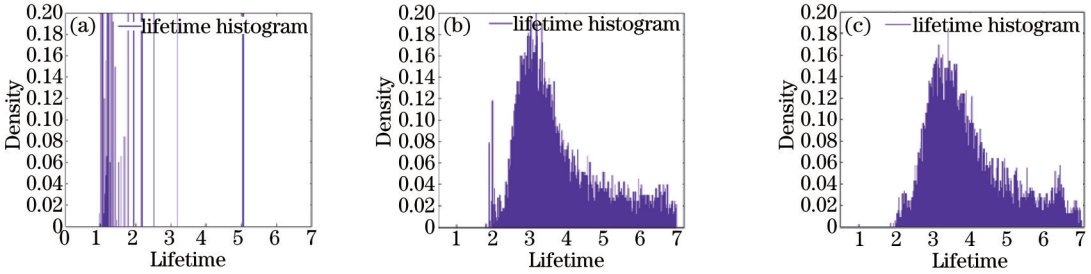

Fig.4 Histograms of lifetime images by (a) rapid lifetime determination; (b) linear least squares method; (c) LM iterative algorithm

References

1 Jo J A, Fang Q, Marcu L. Ultrafast method for the analysis of fluorescence lifetime imaging microscopy data based on the Laguerre expansion technique[J]. IEEE Journal of Selected Topics in Quantum Electronics, 2005, 11(4): 835-845.

2 Liu Chao, Zhou Yan, Wang Xinwei, *et al*.. Fluorescence lifetime imaging microscopy and its progress[J]. Laser & Optoelectronics Progress, 2011, 48(11): 111102.
刘 超, 周 燕, 王新伟, 等. 荧光寿命成像技术及其研究进展[J]. 激光与光电子学进展, 2011, 48(11): 111102.

3 Jo J A, Fang Q, Papaioannou T, *et al*.. Fast model-free deconvolution of fluorescence decay for analysis of biological systems[J]. Journal of Biomedical Optics, 2004, 4(9): 743-752.

4 Pande P, Jo J A. Automated analysis of fluorescence lifetime imaging microscopy (FLIM) data based on the Laguerre deconvolution method[J]. IEEE Transactions on Biomedical Engineering, 2011, 1(58): 172-181.

5 Liu Y, Zhou Y, Liu Y L. A rapid fluorescence lifetime image acquistion method based on time-gated fluorescence lifetime imaging microscopy[C]. IEEE 2nd International Conference on Systems and Informatics, 2014: 808-812.

6 Transtrum M K, Sethna J P. Improvements to the Levenberg-Marquardt algorithm for nonlinear least-squares minimization [OL]. arXiv:1201.5885. http://arxiv.org/abs/1201.5885

7 Marquardt D W. An algorithm for least-squares estimatiosn of nonlinear parameters[J]. Journal of the Society for Industrial & Applied Mathematics, 1963, 11(2): 431-441.

8 Urayama P, Zhong W, Dmitrovsky E, *et al*. A UV-visible-NIR fluorescence lifetime imaging microscope for laser-based biological sensing with picosecond resolution[J]. Applied Physics B, 2003, 5(76): 483-496.

9 Moré J J. Numerical Analysis[M]. Berlin: Springer, 1978: 105-106.

10 Liu Y, Zhou Y, Liu Y L. An active timing controlled-gate fluorescence lifetime imaging microscopy using an ultrafast picosecond diode laser[C]. SPIE, 2014, 9268: 92681S.

11 Liu C, Zhou Y, Wang X W, *et al*.. Lifetime computing algorithms based on exponential pattern retrieve and polynomial fitting in fluorescence lifetime imaging microscopy[C]. SPIE, 2011, 8200: 82000X.

12 Sun Y H, Phipps J, Elson D S, *et al*.. Fluorescence lifetime imaging microscopy: *In vivo* application to diagnosis of oral carcinoma[J]. Optics Letters, 2009, 13(34): 2081-2083.

栏目编辑: 吴秀娟