Estimating Deformation of Neurons in Fluorescent Images

Xianfeng FEI1,*, Takayuki TERAMOTO2, Takeshi ISHIHARA2, and Koichi HASHIMOTO1

1Graduate School of Information Science, Tohoku University, Sendai 980-8579, Japan
2Graduate School of Sciences, Kyushu University, Fukuoka 812-8581, Japan

Received June 5, 2012; final version accepted August 23, 2012

A main challenge in deformation estimation for fluorescent imaging is to decrease the effects of unavoidable random photon shot noise of fluorescence. To solve this open problem, an efficient second-order minimization (ESM) based non-rigid deformation estimation method on fluorescent imaging of neurons is proposed as a visual aid tool for understanding the relationship of neuron activities and behaviors. Because local features, such as corners, lines, arc segments, usually used for deformation estimation, could be compromised by fluorescence noise, global intensity information of all the pixels in a region of interest (ROI) is used as a texture pattern to guide parameterized deformation estimation. By satisfying this principle, three deformation models including affine, homography and Thin Plate Spline (TPS) based on the ESM algorithm are implemented and evaluated. The experimental results illustrate that the homography is the best choice of correctly and robustly estimating parameters for fluorescent deformations under our experimental condition. By using these parameters, the fluorescent intensity in restored image can be measured and analyzed without the disturbance of noise and deformation which can be significant for understanding how nervous system affects and controls animals' behaviors.

KEYWORDS: non-rigid deformation estimation, fluorescent imaging, ESM, homography

1. Introduction

Advances in the natural sciences and medicine have resulted in a shift from exclusively theological or philosophical explanations of human behavior to the understanding that human behavior is controlled by widely distributed neural systems. With the revolution of computer sciences and imaging techniques, scientists are able to study brain function in vivo and begin to yield insights that different neural system regions are responsible for discrete functions. Developments in neuroscience hold great promise for continuing to shape our understanding of mental processes and behaviors that are shared across different animals such as: sensation, perception, motivated behavior (hunger, thirst), control of movement, learning, etc. It also can be benefit in many other ways, including the treatment of psychological disorders, genetic manipulations and drug testing. However, it is difficult to examine the neural activities of all of the neurons in a human-being because the human-being has a huge number of neurons (around 100 billion).

Caenorhabditis elegans (C. elegans) is studied as a model organism for neuroscience because C. elegans is one of the simplest organisms with a nervous system which comprises only 302 neurons. Since its body is transparent, neurons of C. elegans could be observed by imaging. Modernization of imaging techniques, development of new fluorescent tagging proteins, and the rapid growth in computer and informatics technology have resulted in fluorescence microscopy becoming one of the most basic tools used in biological sciences for the visualization of cells. The phenomenon of fluorescence occurs when certain molecules (called fluorophores or fluorescent dyes) absorb light and reach an excited, unstable electronic singlet state, and then decay from the singlet excited state results in fluorescence emission. In fluorescence imaging, specified neurons of C. elegans are labeled with fluorophores. The distribution of fluorescence is observed and captured by photosensitive detectors that measure the intensity of the emitted light and create a digital image of the C. elegans neurons. Our research group has developed an automatic microscope-tracking system which is able to simultaneously monitor and record behaviors of a specified region of moving C. elegans in bright field and their corresponding neuron activities in fluorescent images (Fig. 1) [1, 2].

Because the neural activity during certain behavior can be evaluated by monitoring fluorescent intensity in fluorescence microscopy, our current emphasis is on quantitative analysis of fluorescent images so that the temporal and spatial dynamics of C. elegans neurons can be obtained to quantify the neuron functions during certain behavior. However, C. elegans has a non-rigid body, the motion and the deformation of its body become one of disturbances for measuring fluorescent intensity of neurons through fluorescent imaging.

It is a fundamental yet still open problem in biological imaging to estimate non-rigid deformation with fluorescence microscopy, because there are some limitations in fluorescence imaging that make deformation estimation in
flourescent image challenging. In fluorescence imaging, the quantum nature of light gives rise to a major limitation of any photo detector. Fluorescence occurs when an orbital electron decays into its ground state by emitting a photon of light. The absorption of accumulated photons by a photo detector creates a fluorescent image. The number of photons collected by the photo detector determines the image amplitude at any given point in the image. The intrinsically random nature of photon emission induces an unavoidable noise, known as photon shot noise, to fluorescent imaging [3–5]. The number of photons that are collected by a given detector varies and the probability distribution for $p$ photons in an observation window of length $T$ seconds is known to be Poisson:

$$p(p|\rho, T) = \frac{(\rho T)^p e^{-\rho T}}{p!},$$

where $\rho$ is the rate or intensity parameter measured in photons per second. The intrinsically random nature of photon shot noise makes it impossible to avoid. Consequently, this random noise makes it difficult for us to extract invariant features of observation objects for deformation estimation and restoration.

To overcome the difficulties, a texture-based non-rigid deformation estimation approach is proposed for analysis of fluorescent neuron images of *Caenorhabditis elegans* (*C. elegans*). Our method do not depend on the local features, such as corners, lines, arcs which could be compromised by photon shot noise. The affine matrix, the homography matrix and Thin Plate Spline (TPS) are chosen as the candidates of deformation model. Parameterized deformation models describe the mapping from a region of interest (ROI) in a template image to a corresponding region in an arbitrary image by pixel-wise, therefore, the intensity information of all the pixels in the ROI of the template is used as a texture pattern to guide parameters estimation which counteract the effect of photon shot noise in fluorescent image. To seek a perfect set of parameters to compensate the variation caused by noise and deformation of *C. elegans*, the efficient second-order minimization (ESM) is applied to optimize the parameters. Amongst all standard minimization algorithms, we choose ESM mainly because it has high convergence rate and it can avoid local minima [6, 7]. The proposed three different deformation models are tested and evaluated on the fluorescent image sequences of *C. elegans* neurons. Compared with those original images, the restored images by using estimated deformation parameters are considered to be significantly improved for neural activity observation and analysis.

The rest of this paper is organized as follows. Sections 2 and 3 explains how to employ ESM-based deformation estimation method to search a group of optimized parameters for fluorescent image restoration in detail. Section 4 shows the experiments to evaluate our proposed technique. Section 5 is the conclusion part.

### 2. Deformation Models

As shown in Fig. 2, we suppose that $I^r$ is a $(n \times m)$ reference image. $p^*_i$ denotes the coordinates of a pixel...
Fig. 2. Definition of deformation models. $I^*$ and $I$ represent a reference image and a deformed one. $p^* = [u^*, v^*]^T$ denotes the coordinates of a pixel within the ROI on $I^*$. $p_i = [u_i, v_i]^T$ denotes the coordinates of the corresponding deformed pixel to $p^*$. $w(x)$ is defined as an image deformation operator, thus, deformation between point sets $p_i^*$ and $p_i$ can be written as $p_i \mapsto w(x)(p_i^*)$.

$\begin{align*}
\left[ \begin{array}{c}
{u^*} \\
{v^*} \\
1
\end{array} \right] &= \left[ \begin{array}{c}
{u} \\
{v} \\
1
\end{array} \right] = w(x)(p^*)
\end{align*}$

$(u_i^*, v_i^*) \in 1, 2, \ldots, n \times m$ on $I^*$. $I^*(p_i^*)$ is the intensity of the pixel $p_i^*$. The same definition is applied to a deformed image $I(p_i)$. $p_i = [u_i, v_i]^T$. In order to make our method robust against photon shot noise in fluorescent image, instead of extracting some local features from image which could be compromised by photo shot noise, all the pixels with intensities in our ROI are taken into account as a global texture pattern. A group of optimized parameter is required to describe the texture pattern mapping from a template image $I^*(p_i^*)$ to an arbitrary image $I(p_i)$. $w(x)$ is defined as an image deformation operator, thus, deformation between point sets $p_i^*$ and $p_i$ can be written as $p_i \mapsto w(x)(p_i^*)$. The parameters of the deformation are in the vector $x \in \mathbb{R}^p$, $p$ is the number of dimensions. Affine transformation, homography transformation and Thin plate spline (TPS) are chosen as candidates for $w(x)$ which are implemented to estimate the deformation of $C. elegans$ neurons in fluorescent imaging.

### 2.1 Affine transformation

Geometric contraction, expansion, rotation, shear and translation are all affine transformations, as are their combinations which can be often observed in the fluorescent image sequences of $C. elegans$ neurons [8]. Therefore, an affine transformation matrix is defined as one of our deformation operators:

$$
A = \begin{bmatrix}
1 + a_{11} & a_{12} & a_{13} \\
 a_{21} & 1 + a_{22} & a_{23} \\
0 & 0 & 1
\end{bmatrix}
$$

(2)

$a_{11}$ and $a_{22}$ describe the scale at $u$ and $v$ direction. $a_{12}$ and $a_{21}$ represent the skew at $u$ and $v$ direction. $a_{13}$ and $a_{23}$ are the displacement at $u$ and $v$ direction. Thus, the deformation mapping between pixels in $I^*$ and $I$ can be written as

$$
\begin{bmatrix}
{u_i} \\
{v_i} \\
1
\end{bmatrix} = \begin{bmatrix}
(1 + a_{11})u_i^* + a_{12}v_i^* + a_{13} \\
 a_{21}u_i^* + (1 + a_{22})v_i^* + a_{23} \\
1
\end{bmatrix},
$$

(3)

where $a = [a_{11}, a_{12}, a_{13}, a_{21}, a_{22}, a_{23}]^T$ is the parameter vector of affine transformation model.

### 2.2 Homography transformation

In the field of computer vision, any two images of the same planar surface in space can be related by a homography [9, 10]. A homography is an invertible transformation mapping points and lines from a projective plane to another projective plane which contains three more degrees of freedom than affine transformations. As shown in Fig. 2, an arbitrary point in a plane is projected onto plane $I^*$ as point $p_i^*$ and onto plane $I$ as point $p_i$. The homography mapping between $p_i^*$ and $p_i$ is defined as

$$
sp_i = Hp_i^*,
$$

(4)

where $p_i^* = [u_i^* v_i^* 1]^T$ and $p_i = [u_i v_i 1]^T$. $H$ is so called homography matrix defined as

\[ H = \begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix} \]
We suppose \(s\) in Eq. (4) to be
\[
s = h_{34}u_i^* + h_{32}v_i^* + h_{33},
\]
then the point \(p\) in plane \(I\) is rewritten as
\[
p_i = \frac{H p_i^*}{s} = w(h)(p_i^*),
\]
where \(h = [h_{11}, h_{12}, h_{13}, h_{21}, h_{22}, h_{23}, h_{31}, h_{32}, h_{33}]^T\) is the parameter vector of homography transformation model. \(w(h)\) is the deformation function depending on homography matrix \(H\).

2.3 Thin plate spline

The thin plate spline (TPS) is an effective tool for modeling coordinate transformations [11–13]. The TPS is the 2D generalization of the cubic spline which represents a thin metal sheet on \(u-v\) plane. The process of using TPS as a deformation model is choosing a function \(f\) that minimizes an integral that represents the bending energy of a surface \(E[f]\):
\[
E[f] = \iint \left[ \left( \frac{\partial^2 f}{\partial u^2} \right)^2 + 2 \left( \frac{\partial^2 f}{\partial u \partial v} \right)^2 + \left( \frac{\partial^2 f}{\partial v^2} \right)^2 \right] du dv.
\]
Suppose
\[
f(u, v) = f_0(u, v) + \epsilon \eta(u, v),
\]
where \(\epsilon\) represents parameter and \(\eta(u, v)\) is an arbitrary function which satisfies \(\eta(\pm \infty, v) = \eta(\pm \infty, u) = 0\). To minimize the \(E[f]\), the following equation is required to be satisfied
\[
\frac{dE[f]}{d\epsilon} \bigg|_{\epsilon=0} = 0.
\]
Through a series of deduction, the solution of minimizing the \(E[f]\) can be rewritten as
\[
\Delta^2 f = \left( \frac{\partial^2}{\partial u^2} + \frac{\partial^2}{\partial v^2} \right)^2 f = 0.
\]
Suppose that there is the \(j\)th control points \(c_j = [c_{0j}, c_{1j}, c_{2j}, c_{3j}]^T, j \in 1, k\) on the reference image \(I^*\), thus, with satisfying the Eq. (11), the mapping function \(f_i\) of \(i\)th pixel from \(I^*\) to \(I\) can be constructed as
\[
f_i = a_1 + a_2 u_i^* + a_3 v_i^* + \sum_{j=1}^{k} \omega_j \phi(||p_i^* - c_j||),
\]
where \(||\cdot||\) denotes the usual Euclidean norm and \(a_1, a_2, a_3\) and \(\omega_j\) is a set of mapping coefficients. Our choice for the kernel function \(\phi\) is the radial basis function \(\phi(r) = r^2 \log(r)\). Therefore, given a group of control points \(c_j\) on template image \(p^*\), the deformation model from \(p^*\) to \(p\) can be written as
\[
p_i = w(t)(p_i^*),
\]
where \(t = [a_{11}, a_{12}, a_{13}, a_{14}, a_{21}, a_{22}, a_{23}, a_{24}, \omega_{01}, \omega_{02}, \ldots, \omega_{0k}, \omega_{11}, \omega_{12}, \ldots, \omega_{1k}]^T\) is the parameter vector of TPS model. A nice property of the TPS is that it can be decomposed into a global affine component represented as mapping
3. Estimation of Deformation Parameters

3.1 The ESM iterative minimization

After choosing the potential deformation models, the parameter estimation for these models is proceeded as the diagram shown in Fig. 3. Usually the first image frame is considered to be a reference \( I^*(p_i^*) \) and the rest of image frames \( I(p_i) \) are used as candidates for our deformation estimation experiments. Firstly, the vector of parameters \( x \) for deformation model \( w(x) \) are initialized with a vector of all ones. Then a deformed image under our first guess can be evaluated as:

\[
y(x) = I(w(x)(p_i^*)) - I^*(p_i^*).
\]

The optimized vector of parameters \( x \) will be obtained when the evaluation function reaches its minimum. The ESM is an improved minimization method which has a high convergence rate like the Newton method and does not need to compute the square matrix of second-order partial derivatives [6, 7]. Therefore, the ESM is employed to provide the direction where the intensity differences between transformed image and initial image \( y(x) \) decreases most quickly. The deformation parameters \( x \) are updated iteratively while \( y(x) \) slides down the decreasing most direction until its minimum. Let \( q \) be the total number of pixels in the image and \( p \) be the total number of parameters in the vector \( x \). \( J \) is defined as the \((q \times p)\) Jacobian matrix, i.e. the gradient of the vector \( y(x) \) with respect to the vector \( x \): \( J = \nabla_x y(x) \). Thus, the second-order Hessian matrix of the vector \( y(x) \) can be written as \( M(x_1, x_2) = \nabla^2 y[J(x_1)x_2] \). According to the ESM proposed in [6], instead of using second-order Hessian matrices \( M \), by using the first-order Taylor series approximation of the \( J(x) \) about \( x = 0 \):

\[
J(x) \approx J(0) + M(0, x),
\]

the second-order Taylor series of the vector function \( y(x) \) about \( x = 0 \) can be approximated as:

\[
y(x) \approx y(0) + \frac{1}{2} (J(0) + J(x))x.
\]

For \( x = x_o \), we have:

\[
y(x_o) \approx y(0) + \frac{1}{2} (J(0) + J(x_o))x_o.
\]

Then, the problem consists in finding \( x = x_o \) verifying \( y(x_o) = 0 \). Let \( J_{em} \) be the matrix \( J_{em} = J(0) + J(x_o) \). The evaluation function Eq. (14) has a local or a global minimum in \( x = x_o \) verifying:

\[
x_o = J_{em}^+ y(0),
\]

which means that the deformation parameter vector \( x \) can be updated towards its desired direction by computing the pseudo-inverse of the Jacobians \( J_{em}^+ \).
3.2 Jacobian computation for Affine transformation

The Jacobian for Affine transformation $J_{esma}$ can be written as the product of 2 Jacobians using the chain derivation rule:

$$J_{esma} = \frac{1}{2} (J_{I^*} + J_I)J_a,$$

where $J_{I^*}$ contains the spatial derivatives of the reference image $I^*$ and $J_I$ contains the spatial derivatives of the transformed image $I$. The Jacobian $J_a$ is:

$$J_a = \begin{bmatrix} p_i^T & 0 & \ldots & 0 \\ 0 & p_i^T & \ldots & 0 \\ \vdots & \ddots & \ddots & \vdots \\ 0 & \ldots & 0 & p_i^T \end{bmatrix},$$

which does not depend on $a$ and can be precomputed based on Eq. (3).

3.3 Jacobian computation for homography transformation

Similarly, the Jacobian for homography transformation $J_{esmh}$ can be written as

$$J_{esmh} = \frac{1}{2} (J_{I^*} + J_I)J_h,$$

Based on Eq. (7), $J_h$ can be precomputed as

$$J_h = \begin{bmatrix} p_i^T & 0 & \ldots & 0 \\ 0 & p_i^T & \ldots & 0 \\ \vdots & \ddots & \ddots & \vdots \\ 0 & \ldots & 0 & p_i^T \end{bmatrix}.$$

3.4 Jacobian computation for TPS

Similarly, the Jacobian for TPS transformation $J_{esmt}$ can be written as

$$J_{esmt} = \frac{1}{2} (J_{I^*} + J_I)J_t,$$

Based on Eq. (13), $J_t$ can be precomputed as

$$J_t = \begin{bmatrix} 1 & u_i^* & \ldots & 0 & \phi(||p_i^* - c_1||) & \ldots & \phi(||p_i^* - c_k||) \\ 0 & 0 & \ldots & 0 & 0 & \ldots & 0 \\ 0 & 0 & \ldots & 0 & \phi(||p_i^* - c_1||) & \ldots & \phi(||p_i^* - c_k||) \end{bmatrix}.$$

By using Eqs. (19), (22), and (24), the deformation parameters $a$, $h$, and $t$ can be updated until they are able to transform the deformed images back to the way that the reference image is.

4. Experimental Results

The images to be used in our experiments (dimensions: $222 \times 192$) are explained as in Fig. 4. In order to reduce the effect of dynamic C. elegans, we put a C. elegans in a micro fluidic device. A bright field image of a certain region of C. elegans head as on the left side of Fig. 4 and the corresponding fluorescence image as on the right side are observed to unlock the mystery of the relationship between behaviors and neuron activities. Even with the fluidic device, the obtained fluorescent image sequences of C. elegans neurons still appear unsteady caused by unavoidable photon shot noise and deformations, such as translation, rotation, stretching, shrinking and bending as shown in Fig. 5. These noises and deformation become disturbances for examining the fluorescence intensity of fluorescent protein in C. elegans ’s neurons which are significant information for evaluating how the neural system guide C. elegans’s behaviors. To solve this problem, we conduct the experiments of deformation estimation and restoration on these noisy deformed fluorescent images with three different deformation models based on the ESM.

The experimental results from three deformation models are shown in three sub-figures in Fig. 6. An obtained image sequence is shown on the top row of each sub-figure. The region enclosed by white dots on frame 1 is considered to be the ROI in template image whose size is $150 \times 90$. Instead of the whole image, we merely use the pixels in the ROI for the calculation of deformation estimation. The rest images are used for experiment candidates in which white dots represent the current estimated deformation. The restored images are shown on each bottom row to verify our estimated
deformation parameters. Figure 6(a) shows the results corresponding to the affine transformation model, Fig. 6(b) corresponding to the homography and Fig. 6(c) corresponding to the TPS.

In Fig. 6(a), the results from affine transformation clearly show that our ESM-based affine model successfully exhibit neurons deformation including rotation, shear, transformation and their combinations. We also notice that those white dots used for representation of estimated deformation preserve lines and parallelism (maps parallel lines to parallel lines).

In Fig. 6(b), the results illustrates that our ESM-based homography model not just expresses deformations like rotation, shear, transformation but also reflects partial minor contraction and expansion as shown in frame 10, frame 30, and frame 40.

Figure 6(c) demonstrates the results of deformation estimation by using TPS. The four round white dots inside ROI of frame 0 are chosen as control points. Through the observation of the relative distance between control points and white dots on the boundary, the fact of rigid and non-rigid deformation happening over time can be proved.

In order to evaluate the deformation estimation, we define some sum of squared differences (SSDs) as following:

\[
SSD_c = \sum_{i=1}^{m \times n} ||I(p_i) - I'(p'_i)||^2,
\]

\[
SSD_a = \sum_{i=1}^{m \times n} ||I(w(a)(p'_i)) - I'(p'_i)||^2,
\]

\[
SSD_h = \sum_{i=1}^{m \times n} ||I(w(h)(p'_i)) - I'(p'_i)||^2,
\]

\[
SSD_t = \sum_{i=1}^{m \times n} ||I(w(t)(p'_i)) - I'(p'_i)||^2.
\]

(26)
$SSD_c$ represents the differences between the current image and template image. $SSD_a$ represents differences between the restored images using the affine transformation model and template image. $SSD_h$ and $SSD_t$ are corresponding to the homography and the TPS model. These SSDs are plotted in Fig. 7. $SSD_a$ are drawn in magenta in Fig. 7(a) which shows the differences between current images and template image caused by unavoidable photon shot noise plus deformations, such as rotation, stretching, shrinking and translation. Comparing to the maximum of $SSD_a$ 607.50 and average of $SSD_a$ 239.28, the differences between restored images and template images obtained by using our estimated parameters decrease substantially, for the best scenario, the maximum value down to 48.68 and the average down to 24.94 as shown in Fig. 7(b). The decreases of SSD suggest that using affine, homography or TPS based on ESM is able to estimate the deformations of *C. elegans* neurons against photon shot noise at certain level. Because photon emission in fluorescent imaging is random, we can theoretically prove that using intensity information of all the pixels inside ROI as a texture pattern to estimate deformation can obtain higher signal-to-noise ratio (SNR) than using extracted features, such as corners, edges, shapes and so on.

Comparing the $SSD_a$, $SSD_h$ and $SSD_t$ with each other, firstly, we can see that the results of homography is better than that of affine. This phenomena indicates that the possible deformations of *C. elegans* neurons under our experimental conditions could be better estimated by homography than by affine transformation.
condition is not just translation, rotation and shear also including partial stretching, shrinking and bending. Due to the fact that affine transformation preserves lines and parallelism, homography transformation is more suitable for describing the deformations of *C. elegans* neurons.

Because TPS is a model usually used for non-rigid deformation, the results by TPS is supposed to be better than affine or even better than homography. However, as shown in Fig. 7(b), the SSD in blue are higher than SSD in red and SSD in green. Comparing to the affine model or homography model, the number of parameters of TPS model increases along with the control points number increasing. In our experiments, there are four control points are used which already induces deformation estimation results more sensitive to the photon shot noises.

5. Conclusion and Discussion

In this paper, we have proposed a non-rigid deformation estimation method based on the ESM for fluorescent imaging of neurons. Considering the unique characteristic of fluorescent imaging, i.e., random nature of photon emission, conventional feature extracted method is not appropriate for our problem. Extracted features usually merely come from a small part of image and these features can be compromised by fluorescent noise. Therefore, the information of all the pixels in the ROI of the template is needed to guide deformation estimation. Based on this principle, three deformation models including affine, homography and TPS are implemented and evaluated for fluorescent images of deformed *C. elegans* neurons under the ESM frame. The experiment results indicate that three models are all able to quantify and restore the deformation in fluorescent images of *C. elegans* neurons at a certain level. The best choice under our circumstances is homography transformation model because the affine matrix lacks of parameters for perspective transformation and the TPS is sensitive to the random photon shot noise due to too many parameters. By using our homography parameters, the noisy deformed fluorescent images of *C. elegans* neurons can be restored properly which removes the disturbances of measuring fluorescent intensity of neurons through fluorescent imaging. These results make a significant contribution to the understanding of the basic mechanisms of neural functions.

Fluorescence microscopy has been one of the most basic tools used in biological sciences for the visualization of cells and tissues [14]. With the support of our propose techniques in automated detection and estimation, a thriving fluorescence microscopy results in a shift of biology from qualitative to quantitative.

REFERENCES


