Three-Dimensional Deconvolution of Wide Field Microscopy with Sparse Priors: Application to Zebrafish Imagery

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Abstract—Zebrafish, as a popular experimental model organism, has been frequently used in biomedical research. For observing, analysing and recording labelled transparent features in zebrafish images, it is often efficient and convenient to adopt the fluorescence microscopy. However, the acquired z-stack images are always blurred, which makes deblurring/deconvolution critical for further image analysis. In this paper, we propose a Bayesian Maximum a-Posteriori (MAP) method with the sparse image priors to solve three-dimensional (3D) deconvolution problem for Wide Field (WF) fluorescence microscopy images from zebrafish embryos. The novel sparse image priors include a global Hyper-Laplacian model and a local smooth region mask. These two kinds of prior are deployed for preserving sharp edges and suppressing ringing artifacts, respectively. Both synthetic and real WF fluorescent zebrafish embryo data are used for evaluation. Experimental results demonstrate the potential applicability of the proposed method for 3D fluorescence microscopy images, compared with state-of-the-art 3D deconvolution algorithms.

I. INTRODUCTION

Zebrafish has been used as an important model organism for many kinds of disease research, such as cancer and Parkinson’s disease. Zebrafish is extremely useful to scientists, because this kind of fish is vertebrate with an immune system highly similar to human beings. In addition, zebrafish embryos develop externally and are transparent providing an unparalleled opportunity to study the biology of the immune response. Compared to image analysis of tissue samples from other species, such as mice or humans, where slides with clearly orientated tissue samples of well-defined, standardized thickness would typically form the basis of any subsequent image acquisition and analysis, whole mount in situ staining or immunohistochemistry of zebrafish embryos results in the challenge of having to undertake image analysis in a tissue sample of ill-defined height and orientation.

Zebrafish embryos are observed using light Microscopy, which has been fundamental in advancing our understanding of the molecular and cellular biology of health and diseases. Particularly, WF Fluorescence Microscopy is conventional, and widely used for observing, analysing and imaging specific labelled features of small living specimens. Compared with the confocal microscopy, WF microscopy is much faster and more convenient, which can also record the information of the whole specimen and make the high throughput imaging and large-scale image analysis possible. The current usefulness of zebrafish for high throughput screens is limited due to high variability in signal strength and signal distribution within particular regions of interest of the transparent zebrafish body. These are at least partially due to technical problems whilst embedding the zebrafish embryos for subsequent image analysis but also due to the specific nature of image acquisition in zebrafish.

During the light illumination, both focus and out-of-focus lights are recorded by the Charge-Coupled Device (CCD) camera. To use recorded images for further research, (such as feature detection, cell tracking and cell counting), the out-of-focus light in the z-stack images should be removed first. According to [1], the imaging process using WF or confocal fluorescence microscopy is assumed to be linear and shift invariant. Then, the whole imaging process can be seen as a 3D convolution process. In this paper, we focus on the 3D WF fluorescent images. The 3D fluorescent information of the specimens will be recorded as a z-stack of images. The inverse procedure, i.e., removing the out-of-focus light in z-stack images, is known as 3D deconvolution. The convolution process can be formulated as:

\[
g(x) = n(\sum_{k \in \Omega \subset \mathbb{R}^3} h(k)f(x - k)), \quad (1)
\]

where

- \(g\): observed, blurred image,
- \(h: \mathbb{R}^3 \to \mathbb{R}\): point spread function of the imaging system,
- \(f: \mathbb{R}^3 \to \mathbb{R}\): latent image or ground truth image function,
- \(n: \text{voxelwise noise function},\)
- \(\Omega: \Omega \subset \mathbb{R}^3\) is the support in the specimen domain recorded by the imaging system,
- \(x: 3\text{-tuple of discrete spatial coordinates.}\)

Equation (1) can be written in a short form:

\[
g = n(h * f), \quad (2)
\]
where $\ast$ is the 3D convolution operator.

The convolution kernel $h$ is called Point Spread Function (PSF), which describes the blurring shape of one point light through the light microscope [2]. In this paper, the PSF is calculated using an analytical model if the true PSF is not given, which is called the Variable Refractive index Gibson and Lanni (VRGL) model [3]. The final goal of the deconvolution method is to estimate the best latent image from the observed image with the given PSF. During the imaging process, the fluorescent data are influenced by photon noise, which can be described as a Poisson distribution [4]:

$$p(k) = \frac{\lambda^ke^{-\lambda}}{k!}, k = 0, 1, 2, ...$$

(3)

In this paper, the MAP approach, as a non-linear iteration method, was used for solving many ill-posed problems, such as motion deblurring [19], denoising [20], transparency separation [21] and super resolution [22].

In Fig. 1, the empirical distribution (blue) is the log-probability of the image gradient, and this heavy-tailed distribution is known as the sparse image prior. State of the art estimation strategies for demonstrating the sparse prior model were proposed, including using the Tikhonov-Miller regularizer [23] and the Total Variation (TV) regularization [13], [14], which will be used for comparison in this paper. Different penalty functions were introduced for the gradient filter, such as $f_1(x) = |\nabla x|$ for the TV regularization method and $f_1(x) = |\nabla x|^2$ for the TM regularization method [13].

The TV regularization term was applied to 3D deconvolution for microscopy data [13]. In [14], the TV regularization term was presented for blind iterative deconvolution by updating both the latent image function and the PSF simultaneously.

The Hyper-Laplacian distribution has been applied to 2D non-blind deconvolution [24]. In [18], the Hyper-Laplacian distribution was modified with the Gaussian noise model, and a lookup table (LUT) was developed for improving the computational speed. In [17], both global and local priors were introduced for solving the motion deblurring problem from a single image. For the global prior, they presented two piece-wise continuous functions to fit the heavy-tailed distribution. The local prior was used for suppressing ringing artifacts. Our local prior part is similar to the method in [17].
III. DECONVOLUTION FRAMEWORK

In this section, a Maximum a-Posterior algorithm with sparse priors is presented for estimating the best latent image \( f \) given the blurred image \( g \) and the PSF \( h \). According to the Bayes Rule:

\[
p(f|g,h) = \frac{p(g|f,h)p(f)}{p(g)} \propto p(g|f,h)p(f),
\]

where \( p(f) \) is a prior about \( f \), \( p(g|f,h) \) can be seen as a Poisson distribution with mean \((f*h)(x)\), and Equation (3) can be rewritten as:

\[
p(g(x)|f,h) = \frac{(f*h)(x)^{g(x)}e^{-(f*h)(x)}}{g(x)!}.
\]

The sparse image priors will be discussed in the following subsections.

A. Sparse Image Priors

In this paper, to simultaneously address the ill-posed nature of the problem and ringing artifacts reduction when restoring the latent image \( f \), the prior \( p(f) \) is decomposed into two components, including a global prior \( p_g(f) \) [18] and a local prior \( p_l(f) \) [17]:

\[
p(f) = p_g(f)p_l(f).
\]

1) Global Prior \( p_g(f) \): The heavy-tailed distribution of the gradient in the WF fluorescence microscopy z-stack is explained well by the Hyper-Laplacian distribution [18]. In Fig. 1, it can be seen that the best approximation for the latent image \( f \), the prior \( p(f) \) is defined as:

\[
p_g(f) \propto \prod_{i \in \Omega \subset \mathbb{R}^3} e^{-\tau|\nabla f_i|^\alpha}, \quad (0 < \alpha < 1),
\]

where \( \tau \) is the positive rate parameter, and \( \nabla \) is the 3D first order derivative filter.

2) Local Prior \( p_l(f) \): Besides the global prior, a local prior with mask is used for reducing ringing artifacts, which was introduced first in a motion deblurring problem [17]. In this paper, we design a similar local prior for 3D deconvolution, but the difference is that we use a triangle threshold method [25] to determine the smooth region in the z-stack images automatically. It is indicated in [17] that the smooth region in the image should also be smooth after restoration, and this local prior can suppress ringing artifacts effectively in both the smooth regions and the texture regions. Due to the inaccurately generated PSF, some ringing artifacts will appear near the boundary and the edges after a number of iterations during the restoration in the frequency domain. Similar to [17], the error of the gradient between the estimated image and the blurred image in the smooth area is defined to follow the Normal distribution with zero mean:

\[
p_l(f) = \prod_{i \in \Phi} N(\nabla f_i - \nabla g_i|0,\sigma),
\]

where \( \Phi \) denotes the smooth regions in each frame of the z-stack images, and the smooth regions \( \Phi \) are shown in Fig. 2. Even though this local prior is only defined in the smooth regions, it can globally reduce ringing artifacts due to the effect of the PSF during the restoration [17]. We define a mask function, \( M(x) \), to describe the smooth regions \( \Psi \) in the z-stack images. The triangle threshold algorithm [25] is applied to determine the threshold automatically, and \( \Psi \) indicates the regions where \( M(x) = 1 \):

\[
M(x) = \begin{cases} 
0, & g \geq \text{Threshold} \\
1, & \text{else}.
\end{cases}
\]

B. Optimization

After defining the two priors, Equation (4) becomes:

\[
p(f|g,h) \propto p(g|f,h)p_g(f)p_l(f).
\]

Instead of maximizing the likelihood probability of Equation (10), the negative log-likelihood function, \( J(f) \), is minimized. In the function \( J(f) = -\log(p(g|f,h)) \) is defined as \( J_{\text{MLEM}}(f) \).

\[
J(f) = -\log(p(g|f,h))
\]

\[
\propto -\log(p(g|f,h)) - \log(p_l(f))
\]

\[
= -\log(p(g|f,h)) - \log(p_g(f)) - \log(p_l(f))
\]

\[
= J_{\text{MLEM}}(f) + \int_{\Sigma} \lambda_g|\nabla f|^\alpha \, dx
\]

\[
+ \int_{\Phi} \lambda_l|\nabla f - \nabla g|^2 \, dx,
\]

where \( J_{\text{MLEM}}(f) \) is redefined as:

\[
J_{\text{MLEM}}(f) = \int_{\Sigma} (f*h) - g(x) \log(f*h)(x)
\]

\[
+ \log(g(x)) \, dx
\]

\[
\propto \int_{\Sigma} (f*h) - g(x) \log(f*h)(x) \, dx.
\]
The derivative of $J$ with respect to $f$ is calculated and set to zero. Then, we obtain a regularized version of the MLEM updating scheme:

$$f_{k+1}(x) = f_k(x) / \left(1 - \lambda_2 \Psi_g - \lambda_1 \Psi_l\right) \cdot \frac{g(x)}{(f_k * h)(x)}, \quad (13)$$

where $\Psi_g = div\left(\frac{\nabla f_k}{\|\nabla f_k\|^2 - \alpha}\right)$ and $\Psi_l = div\left((\nabla f_k - \nabla g)M(x)\right)$ are the regularization terms; $\lambda_2, \lambda_1$ are the regularization parameters; $div$ is the divergence, e.g., $div(f) = \nabla \cdot f$. The iteration process in Equation (13) is implemented in the frequency domain with Fast Fourier Transform (FFT), which extremely improves the computational speed. To find a stop criterion, we calculate the Root Mean Squared Error (RMSE) [14], [15] value in each iteration between the updated latent image and the ground truth image, so we calculate the RMSE value between updated latent image and blurred image. Then, the iteration will be stopped until the changes of the RMSE value is smaller than a small constant value.

C. PSF Modelling

In the updating scheme (13), we assume the PSF ($h$) is known. However, in the real situation, the true PSF is not given, so we use the VRGL model to produce the PSF. Compared with 2D deblurring, solving the 3D deconvolution problem is more complex and time-consuming. To obtain better results, the precise PSF is needed to avoid losing some useful information during deconvolution process. There are usually three ways to estimate the PSF, namely experimental, analytical and computational methods [26]. Using the experimental method to obtain the PSF usually results in a very poor Signal-to-Noise Ratio (SNR) [7]. In the computational method, such as blind deconvolution, both of the PSF and the latent image are calculated based on the blurred image, and the optimal result is seriously biased by the initial guess of the PSF. An analytical method, i.e., the VRGL model, is used for modelling the PSF in this paper. The VRGL model is designed to estimate the PSF of the microscopy system, which captures the aberration that caused due to variation of refractive index within the thick specimen. The comparison of different PSF models is beyond the scope of this paper, and more details of the VRGL model are explained in [3].

IV. EXPERIMENTS AND RESULTS

To demonstrate the effectiveness of the proposed approach, experiments are conducted using three different datasets, including two synthetic datasets and one real WF Fluorescence Microscopy Zebrafish Embryo dataset. All of the results are evaluated using the Root Mean Squared Error (RMSE) [14], [15], Peak Signal-to-Noise Ratio (PSNR) and Normalized Mean Integrated Squared Error (NMISE). The MAP with global Hyper-Laplacian (HL) and the MAP with Hyper-Laplacian and Local Mask (HL+Mask) are tested, compared with TV and TM regularization methods.

A. Synthetic Data

Two groups of synthetic data are used for testing, including the Hollow Bar and the Hela Cell Nucleus dataset. For the TV and TM regularization methods, the RMSE lines converge after about 120 iterations.

1) Hollow bar: The synthetic hollow bar data are collected from http://bigwww.epfl.ch/deconvolution/?p=bars. The ground truth data are blurred first using a theoretical WF microscopic PSF, and then corrupted by both Gaussian noise and Poisson noise with the signal-to-noise ratio (SNR) of 15dB. Voxel volumes of this dataset are $256 \times 256 \times 128$. Parameters of the synthetic PSF include Numerical Aperture $NA=1.4$, spherical aberration $W = 0$, wavelength $\lambda = 500nm$, spatial resolution $\Delta r = 100nm$ and Axial resolution $\Delta z = 200nm$. The best results for TM, TV regularization and proposed method are achieved with regularization parameters $\lambda_{TM} = 3 \times 10^{-7}$, $\lambda_{TV} = 0.001$, $\lambda_g = 0.05$, $\lambda_l = 10^{-8}$ and $\alpha = 1/3$. Results using different methods after 120 iterations are shown in Fig. 3, which illustrates that the two proposed methods have improved the results of TM and TV. The result using the TM regularization is over smooth after 100 iterations, and the regularization term will become larger and larger during iterations. The result of HL+Mask is a little sharper than that of HL. In Fig. 3, the tendency of the RMSE using
4 different methods is shown. The TV and TM methods have already converged after 100 iterations, and the RMSE lines of the two proposed methods are much lower than those of the other two methods after 50 iterations.

2) HeLa Cell Nucleus: The HeLa Cell Nucleus dataset was first used in [14], and the ground truth data were generated using an online simulation tool [27]. The data are corrupted by a WF PSF without aberration, and then the Poisson noise is added. The SNR of the final blurred data is 1.61. The PSF is generated with Numerical Aperture NA = 1.4, wavelength $\lambda = 530\text{nm}$, spatial resolution $\Delta r = 64.5\text{nm}$ and Axial resolution $\Delta z = 160\text{nm}$. For this dataset, $\lambda_{TM} = 10^{-5}$, $\lambda_{TV} = 0.005$, $\lambda_g = 0.01$ with $\alpha = 1/3$, $\lambda_l = 10^{-7}$ with $\alpha = 1/3$ are chosen for testing TM, TV, proposed HL and proposed HL+Mask methods, respectively. After 120 iterations, the results and the RMSE are shown in Fig. 4.

The results shown in Fig. 4 are based on 4 different methods after 120 iterations. It can be seen easily that more out-of-focus light is reduced using the proposed methods. The TV regularization method can preserve the edges of the image, but the result is over smooth in the whole image. Fig. 5 illustrates that all of the 4 methods converge after about 60 iterations. After convergence, the RMSE of the results using the proposed methods is lower than that of the TV and TM methods. In Table 1, scores of proposed methods using two other performance metrics, PSNR and NMISEC, are also the best, compared with TV and TM regularization methods.

B. Zebrafish Embryo Data

We collected fluorescent light microscopy images of entire living vertebrates (zebrafish up to 5 days post fertilisation) that contain information on single immune cell types and pathogens in the whole organism. The WF fluorescent zebrafish embryo dataset was recorded by In Cell Analyzer 2000, and the Microscopy type is fluorescence with Numerical Aperture NA = 0.1, Wavelength $\lambda = 490\text{nm}$, Spatial resolution $\Delta r = 3.7\text{µm}$, Axial resolution $\Delta z = 20\text{µm}$, magnification $=2.0$, focal length $=100\text{µm}$. The stack size is $2048 \times 2048 \times 41$. Parameters for this dataset include $\lambda_{TM} = 5 \times 10^{-5}$, $\lambda_{TV} = 0.01$, $\lambda_g = 0.05$ with $\alpha = 1/3$, $\lambda_l = 10^{-6}$ with $\alpha = 1/3$. There is no corresponding ground truth data for the recorded zebrafish images, so quantitative scores using performance metrics of the final results are not calculated. The qualitative results are shown in Fig. 6, and in Fig. 7, we zoom in on a specific region of the results to get more details. As expected, the proposed HL and HL+Mask methods produce improved results, compared with TM and TV.

V. Conclusion

In this paper, we have proposed a unique deconvolution method for 3D WF microscopy data using the MAP approach with sparse priors. The novelty of the method is that we use the Hyper-Laplacian distribution as a sparse prior to describe the log-gradient distribution, and a local mask is applied to 3D deconvolution for reducing the ringing artifacts. The Hyper-Laplacian distribution is much closer to the log-gradient distribution than other models, especially for WF z-stack images. Results on both synthetic data and real WF fluorescence data from zebrafish embryos show that the proposed methods have potential applicability for 3D WF microscopy images, compared with other methods. Limitations of the proposed methods are that it takes hundreds iterations to obtain a satisfactory result, which is time consuming, and the parameters are not adaptive. Future work will address the issue of the best way to determine the suitable parameters, and

![Fig. 4: The HeLa cell dataset results using 4 different methods after 120 iterations.](image)

![Fig. 5: RMSE results for Hela Cell synthetic data. Blue: TM, Green: TV, Black: Proposed HL, Red: Proposed HL+Mask](image)

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<th>HeLa</th>
<th>RMSE</th>
<th>PSNR</th>
<th>NMISEC</th>
<th>TV</th>
<th>HL</th>
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TABLE I: Evaluation using 3 different performance metrics

![Image](image)
also to improve the computational speed for high throughput image analysis.

REFERENCES


