論 文 の 内 容 の 要 旨

論文題目　Blind Deconvolution of 3D Fluorescence Microscopy using Depth-variant Asymmetric PSF
(奥行き可変非対称PSFを用いた3次元蛍光顕微鏡画像のブラインドデコンボリューション)

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Three-dimensional (3D) fluorescence wide-field microscopy (WFM), is widely used in biomedical research since 3D observation of cellular structure is possible with high contrast. The 3D WFM images are obtained by piling up 2D images of different in-focus planes. However, there is a problem that an out-of-focus blur obscures the in-focus detail. Restoration of a clear object image of 3D WFM has been challenged.

For the purpose, this thesis gives importance to implement practical deconvolution and improving accuracy of PSF estimation. The practical deconvolution means an implementation of the deconvolution that has an appropriate imaging model with a convergence guarantee on a PC. The practical deconvolution enables to estimate accurate object image with computational cost expectation.

Also, improving accuracy of PSF estimation in this thesis is directly connected to reflect WFM blur properties. WFM lens is designed to focus the specimen plane right above a microscopy stage with the refractive index of immersion layer. However, the 3D WFM observes planes inside specimen; the refractive index of specimen causes peculiar problems. First, the blur varies according to depth. As WFM focuses to deeper plane of specimen, refractive index mismatch between immersion and specimen layer causes severer blur. This causes the elongation in the axial axis. Second, the PSF is specimen-dependent. Even if the pre-measured point spread function (PSF) is given, the PSF is not accurate since the refractive index and the focal distance of the point-like object for pre-measuring and those of the actual specimen are different, which indicates that PSF pre-measurement is useless. Moreover, even if it is in perfect imaging conditions, an imperfection of lens causes asymmetric blur. Based on practical deconvolution for WFM, this thesis solves these problems through depth-variant algorithm, blind deconvolution and asymmetric PSF model utilization. Namely, this thesis covers the main causes of blur in WFM imaging condition and optical system design.

First work proposes depth-variant deconvolution. For practical deconvolution, generalized expectation maximization algorithm is introduced and applied to WFM under depth-variant imaging model. PSF is estimated by fitting parameters of the theoretical PSF to the pre-measured PSF. From comparison among existing deconvolution methods, it is proven that the GEM has superior and practical deconvolution method. A normalized correlation coefficient (COR) value between true object image and deconvolution image is used for evaluation. However, the deconvolution result shows that the axial blur is not perfectly suppressed in actual image; the author supposed that the remained blur is caused by the inaccuracy of the pre-measured PSF.

Second work focuses the inaccuracy of the pre-measured PSF. The inaccuracy occurs since the refractive index and the focal distance vary when users switch the point-like object for pre-measuring into the actual specimen. In this work, I propose the blind deconvolution - the estimation parameters of equation based PSF from the observed image. While the first work fits parameters to the pre-measured PSF, the second work fits parameters to the obtained PSF from the observed image. Experiments are implemented using the opened data of micro-bead image, which enables to compare performances to previous algorithms. Since blind deconvolution does not have the true image, the COR cannot be used as evaluation indicators. The micro-bead has determined diameter and hollow sphere shape; therefore, the diameter and the relative contrast between shell and hollow inside can be used for performance indicators. From the indicators, the author proves that the second work completely suppresses the remained blur. However, despite the symmetric sphere shape of the micro-bead, the deconvolution result shows the asymmetric shape.

Third work focuses the asymmetric result. The asymmetric result alludes that the blur has an asymmetric shape. The author proposes a blind deconvolution using depth-variant asymmetric PSF. While the theoretical PSF model used in the first and the second work considers axial asymmetric but x-y symmetric blur that specimen causes, the third work utilizes xyz asymmetric PSF that not only the specimen but also lens aberrations cause. In this work, the author devised new performance indicators to evaluate symmetry of deconvolution results. Standard deviation values of diameter and shell intensities along x, y and z axis are used as new indicators. This work compares performances by indicators that are used in the second work and the new indicators, which are conducted using the opened data of micro-bead image. Qualitatively, the deconvolution result images show that asymmetric distortions are removed. Quantitatively, while transversal, axial diameter error and contrast in existing method are 236nm, 477nm and 88%, ours have 180nm, 84nm and 98% values. These results show the third work generates superior performances. Also, standard deviation values of diameter and relative contrast for symmetry evaluation are 143nm and 4.4%, which are better than 198nm and 12.8% values in second work and show that the asymmetry is corrected. Finally, with deconvolution results, the author summarizes deconvolution quality, computational cost according to deconvolution methods, which would be a helpful guide for 3D WFM users.

Differences in performance among three works can basically explain different characteristics of WFM blur. Reflecting characteristics of blur is significantly important key for determining the quality of the deconvolution result.