Three-dimensional localization of defocused fluorescence based on deep learning

Gwiyeong Moon, Taehwang Son, and Donghyun Kim
School of Electric and Electronic Engineering
Yonsei University
Seoul, 120-749, South Korea,
kimd@yonsei.ac.kr

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Single molecule localization microscopy (SMLM) emerges as a method applicable to many biomedical engineering issues, for example, for analysis of molecular dynamics and nanoscale tracking. Also, studies based on three-dimensional (3D) localization have drawn significant attention as well as accurate and fast algorithms for retrieval of 3D information. Among many methods of 3D localization microscopy, defocused imaging utilizes point spread function (PSF) of an emitter to obtain 3D information from single 2D image. However, PSF of emitter positioned at out-of-focus plane spreads because of diffraction, the pattern of which would overlap in case of a high concentration of emitters, making retrieval of 3D information extremely difficult. In this study, we investigate a method for retrieval of 3D information from defocused 2D wide field images of high-density molecules based on deep learning. Fully convolutional network (FCN) and convolutional neural network (CNN) were employed for lateral and axial localization, respectively. Instead of methods based on shifting kernel in an entire image [1], the image was used as an input to FCN for lateral localization by reducing the complexity of analysis. With CNN, axial localization was performed in an image which is cropped around the lateral position obtained from FCN. Images of a single fluorescent bead were acquired and augmented by shifting an image and adding noise to train the neural network. Brownian motion of fluorescent beads was taken to test our 3D localization method. It is expected that our approach can be widely applied to various molecular localization and tracking in high-density and low SNR condition.

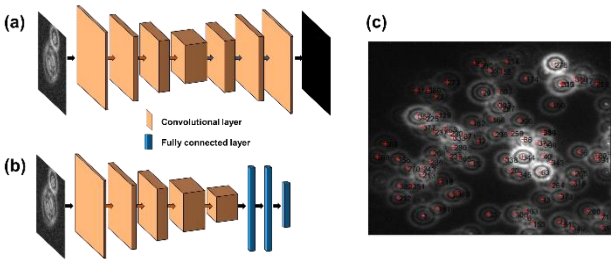

Figure 1. Schematics: (a) FCN for lateral localization and (b) CNN for axial localization. (c) Result of 3D localization of fluorescent beads.

[1] J. M. Newby, A. M. Schaefer, P. T. Lee, M. G. Forest and S. K. Lai, “Convolutional neural networks automate detection for tracking of submicron-scale particles in 2D and 3D,” *Proceedings of the National Academy of Sciences*, **115**, 9026-9031 (2018).