Convolutional Neural Network Denoising in Fluorescence Lifetime Imaging Microscopy (FLIM): Extended Abstract

Varun Mannam*[1], Yide Zhang[1,2], Xiaotong Yuan[1], Takashi Hato[3], Pierre C. Dagher[3], Evan L. Nichols[4], Cody J. Smith[4], Kenneth W. Dunn[3], and Scott Howard[1]

[1]Department of Electrical Engineering, University of Notre Dame, Notre Dame, IN 46556, USA
[2]Caltech Optical Imaging Laboratory, Andrew and Peggy Cherng Department of Medical Engineering, California Institute of Technology, Pasadena, CA 91125, USA
[3]Department of Medicine, Division of Nephrology, Indiana University, Indianapolis, Indiana 46202, USA
[4]Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556, USA

1. CONVOLUTIONAL NEURAL NETWORKS (CNN) FOR REAL-TIME DENOISING IN AN INSTANT FLIM SYSTEM

Fluorescence lifetime imaging microscopy (FLIM) is a powerful technique in biomedical research which provides enhanced molecular contrast in addition to conventional fluorescence imaging. However, traditional FLIM systems are limited by their slow processing speed, low signal-to-noise ratio (SNR), and expensive and challenging hardware setups. Previously, we have demonstrated a novel instant FLIM system[1] based on analog signal processing for fast data acquisition as shown in Figure 1 (a). This Instant FLIM system provides high SNR using high-efficiency pulse-modulation, and cost-effective implementation by utilizing off-the-shelf radio-frequency components like phase shifters, mixers and low-pass filters (LPFs). Moreover, due to analog processing, we are able to measure the intensity, lifetime, and phasor plots for 2D, 3D, or 4D imaging in *in vivo* and *ex vivo* FLIM measurements simultaneously. Figures 1 (b) and (c) show intensity and lifetime images of zebrafish embryos

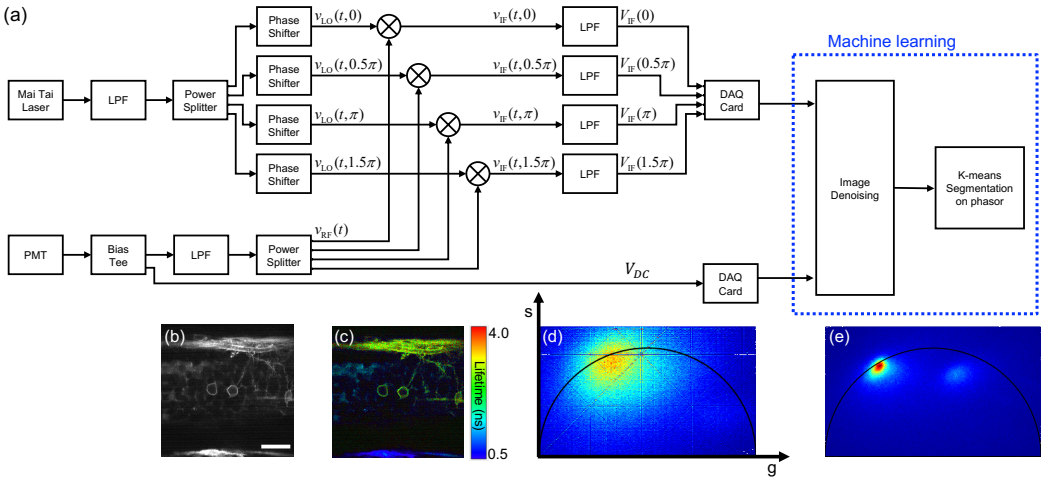

Figure 1. Block diagram of the denoising instant FLIM. Diagram showing how analog signals are processed in instant FLIM followed by the machine learning section, which contains image denoising and K-means segmentation (a). The intensity of *in vivo* zebrafish embryo acquired with a custom-built instant FD-FLIM setup (b), fluorescence lifetime (c), 3D phasor stack before and after denoising (d) and (e) respectively. Scale bar: 20 μm.

(*in vivo*), respectively, that are captured using the instant FLIM setup. Figure 1 (d) shows the spread-out 3D stack phasor diagram due to the noisy FLIM measurements. Traditionally the noisy FLIM measurements pass through either a mean or median filter[2] multiple times that enhances the phasor. In this paper, we demonstrate image denoising using the pre-trained deep learning model[3] (using fluorescence intensity images) on the instant FLIM measurements to provide an accurate phasor. Figure 1 (e) shows the enhanced phasor when the noisy FLIM measurements are passed through deep learning-based image denoising. Also, our denoising method is computationally faster ($<$ 80ms). Segmentation using the enhanced phasor is explained in the next section.

*e-mail: vmannam@nd.edu

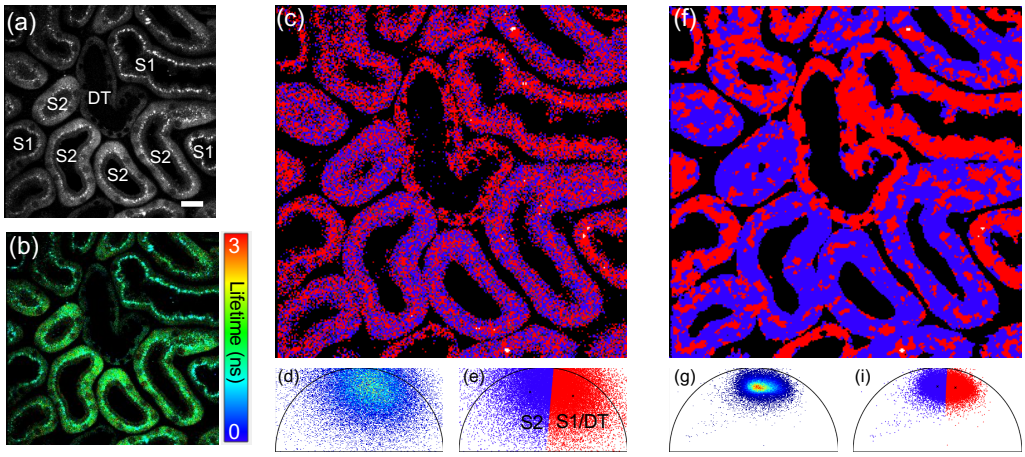

Figure 2. K-means clustering applied to phasor data of a living mouse kidney acquired with a commercial FD-FLIM system.[4] Two-photon intensity image along with microtubules marking either $S1/DT$ or $S2$ (a), fluorescence lifetime image (b), the overlap of both segments (c) and (f), phasor plot (d) and (g), K-means clustering on phasors (e) and (i) before and after image denoising respectively. The red color and blue color represent $S1/DT$ (short lifetime) and $S2$ (long lifetime) microtubules, respectively. Scale bar: 20 μm.

2. IMPROVED *IN VIVO* FLIM PHASOR SEGMENTATION USING CNN DENOISING

Image denoising and segmentation is demonstrated in Figure 2 where (a) and (b) show the intensity and fluorescence lifetime, respectively, for an *in vivo* mouse kidney (male C57BL/6J mice at 8–10 weeks of age, obtained from The Jackson Laboratory) acquired with a commercial digital FD-FLIM system.[4] We labeled (as a ground truth and verified[4]) the intensity image with the mouse distal tubules (DT) and proximal tubules (upstream $S1$ and downstream $S2$) with distinct metabolic signatures, which can be resolved with FLIM phasors. Furthermore, $S1$ and DT are remarkably similar in FLIM signatures despite their morphological difference; therefore, we consider only to categorize $S1/DT$ and $S2$ as two clusters. Figure 2 (c) shows the phasor labeled of the two clusters into a single image generated from the noisy phasor in (d) followed by an unbiased and unsupervised K-means clustering segmentation method. Figure 2 (e) shows the K-means clustering result of the noisy phasor, where the red and blue color pixels in the phasor indicate the $S1/DT$ and $S2$ microtubules, respectively. Also, the upstream proximal tubules have a shorter lifetime (right side of the phasor) compared to the downstream tubules, which has a longer lifetime (left side of the phasor). From the overlapping clusters in Figure 2 (c), it is hard to identify these tubules accurately. To address this issue, we use deep learning based image denoising on the FLIM measurements to enhance the phasor, as shown in Figure 2 (g). K-means clustering on the enhanced phasor, shown in Figure 2 (i), provides accurate segmentation results. From the segmented images after denoising, the phasor labeled image is shown in Figure 2 (f), where the clear indication of dominant red and blue color tubules are mapped to $S1/DT$ and $S2$ proximal tubules, respectively. Hence, the proposed deep learning-based workflow provides fast and accurate automatic segmentation of fluorescence images using instant FLIM. The instant FLIM system, along with the denoising and segmentation methods, are especially useful if the FLIM measurements are noisy, and the clustering can effectively enhance the detection of biological structures of interest in biomedical imaging applications.

REFERENCES

[1] Zhang, Y., Guldner, I. H., Nichols, E. L., Benirschke, D., Smith, C. J., Zhang, S., and Howard, S. S., "Instant FLIM enables 4d *in vivo* lifetime imaging of intact brains," *bioRxiv* (2020).

[2] Digman, M. A., Gratton, E., Marcu, L., French, P., and Elson, D., "The phasor approach to fluorescence lifetime imaging: Exploiting phasor linear properties," *Fluorescence Lifetime Spectroscopy and Imaging* , 235–248 (2014).

[3] Zhang, Y., Zhu, Y., Nichols, E., Wang, Q., Zhang, S., Smith, C., and Howard, S., "A poisson-gaussian denoising dataset with real fluorescence microscopy images," in [*Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*], 11710–11718 (2019).

[4] Zhang, Y., Hato, T., Dagher, P. C., Nichols, E. L., Smith, C. J., Dunn, K. W., and Howard, S. S., "Automatic segmentation of intravital fluorescence microscopy images by K-means clustering of FLIM phasors," *Optics Letters* **44**(16), 3928–3931 (2019).