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Deep learning augmented microscopy: a faster, wider view, higher resolution autofluorescence-harmonic microscopy

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Abstract

Deep learning enables bypassing the tradeoffs between imaging speed, field of view, and spatial resolution in autofluorescence-harmonic microscopy.

Label-free nonlinear optical microscopy is an emerging technique for probing biological structures and functions without exogenous labels or dyes. Thus, it provides an attractive solution to gain biological insights without perturbing the native states of biological samples and processes. For example, vibrational spectroscopic imaging techniques¹, such as anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), allow quantifying biomolecules by measuring the molecular vibration spectra in living cells and tissues. Two-photon autofluorescence (2PA) of flavin adenine dinucleotide (FAD) and three-photon autofluorescence (3PA) of nicotinamide adenine dinucleotide (NADH) permit noninvasive monitoring of metabolic activities². Second-harmonic generation (SHG) microscopy provides morphological and functional characterization of anisotropic biological structures, such as collagen³, muscle⁴, and microtubules⁵. Third harmonic generation (THG) microscopy enables elucidating on cellular and tissue organizations by probing intra- and extracellular membranes, and extracellular matrix structures⁶. Recent advances in simultaneous label-free autofluorescence- multiharmonic (SLAM) microscopy further expands the nonlinear optical microscopy's utility in intravital imaging⁷ and slide-free, stain-free histopathology^{8,9}.

Despite these advances, the implementations of all nonlinear optical microscopy techniques rely on laser scanning. This makes it challenging to simultaneously achieve a wide field of view (FOV), high spatial resolution, and fast imaging speed with sufficient signal-to-noise ratio (SNR) in the measurement. In this work by Shen et al.¹⁰, a novel deep learning augmented microscopy framework is developed to overcome the physical tradeoffs. The proposed “deep learning autofluorescence-harmonic microscopy” (DLAM) is demonstrated on human pathological tissues for multimodal imaging, including 2PA of FAD, SHG and 3PA of NADH, with enhanced spatial resolution and much reduced acquisition time. This advancement may find broad utilities in the studies of biology and neuroscience.

Broadly speaking, DLAM adds to the rapid-growing list of deep learning augmented microscopy techniques¹¹, which overcome different aspects of physical limitations by combining novel instrumentation and deep learning. For example, strategies have been developed to first acquire low-SNR images at high speed and low light exposure and later enhance the SNR by deep learning to alleviate photo-damages in fluorescence microscopy¹² and SRS microscopy¹³. Deep learning-based super-resolution reconstruction has been demonstrated to bypass the limitation of FOV¹⁴. Data-efficient acquisition schemes by deep learning have been developed for multi-shot quantitative imaging techniques, such as Fourier ptychographic microscopy¹⁵, single molecule localization microscopy¹⁶, and structured illumination microscopy¹⁷. We envision

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this deep learning augmented approach may fundamentally push the imaging limits and ultimately revolutionize the field of microscopy.

Conflict of interest

The authors declare no competing interests.

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References

- Cheng, J. X. & Xie, X. S. Vibrational spectroscopic imaging of living systems: an emerging platform for biology and medicine. *Science* **350**, aaa8870 (2015).
- Heikal, A. A. Intracellular coenzymes as natural biomarkers for metabolic activities and mitochondrial anomalies. *Biomark. Med.* **4**, 241–263 (2010).
- Cicchi, R. et al. From molecular structure to tissue architecture: collagen organization probed by SHG microscopy. *J. Biophotonics* **6**, 129–142 (2013).
- Plotnikov, S. V. et al. Characterization of the myosin-based source for second-harmonic generation from muscle sarcomeres. *Biophysical J.* **90**, 693–703 (2006).
- Dombeck, D. A. et al. Uniform polarity microtubule assemblies imaged in native brain tissue by second-harmonic generation microscopy. *Proc. Natl Acad. Sci. USA* **100**, 7081–7086 (2003).
- Weigelin, B., Bakker, G. J. & Friedl, P. Third harmonic generation microscopy of cells and tissue organization. *J. Cell Sci.* **129**, 245–255 (2016).
- You, S. X. et al. Intravital imaging by simultaneous label-free autofluorescence-multharmonic microscopy. *Nat. Commun.* **9**, 2125 (2018).
- You, S. et al. Slide-free virtual histochemistry (Part I): development via nonlinear optics. *Biomed. Opt. Express* **9**, 5240–5252 (2018).
- You, S. et al. Slide-free virtual histochemistry (Part II): detection of field cancerization. *Biomed. Opt. Express* **9**, 5253–5268 (2018).
- Shen, B. L. et al. Deep learning autofluorescence-harmonic microscopy. *Light: Sci. Appl.* **11**, 76 (2022).
- Tian, L. et al. Deep learning in biomedical optics. *Lasers Surg. Med.* **53**, 748–775 (2021).
- Weigert, M. et al. Content-aware image restoration: pushing the limits of fluorescence microscopy. *Nat. Methods* **15**, 1090–1097 (2018).
- Lin, H. N. et al. Microsecond fingerprint stimulated Raman spectroscopic imaging by ultrafast tuning and spatial-spectral learning. *Nat. Commun.* **12**, 3052 (2021).
- Wang, H. D. et al. Deep learning enables cross-modality super-resolution in fluorescence microscopy. *Nat. Methods* **16**, 103–110 (2019).
- Xue, Y. J. et al. Reliable deep-learning-based phase imaging with uncertainty quantification. *Optica* **6**, 618–629 (2019).
- Nehme, E. et al. Deep-STORM: super-resolution single-molecule microscopy by deep learning. *Optica* **5**, 458–464 (2018).
- Jin, L. H. et al. Deep learning enables structured illumination microscopy with low light levels and enhanced speed. *Nat. Commun.* **11**, 1934 (2020).

