Frontiers in Optics + Laser Science 2023 (FiO/LS) © Optica Publishing Group 2023

Enhanced Volumetric Reconstruction in Fourier Light-Field Microscopy through Denoising-Sparse Decomposition

Zhi Ling,[1,2,3] Wenhao Liu,[1] Biagio Mandracchia[4,5], Jessica Hou[6], Shuyi Nie[3,6] and Shu Jia[1,3,*]
1 The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332 USA
2 George W. Woodruff School of Mechanical Engineering, Georgia Institute of Technology, Atlanta, GA, 30332 USA
3 Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA 30332 USA
4 Centro Nacional de Microbiología/ISCIII, Majadahonda, Madrid, Spain
5 ETSI Telecomunicación, Universidad de Valladolid, Valladolid, Spain
6 School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332 USA
**shu.jia@gatech.edu*

Abstract: We propose a denoising-sparse decomposition method for Fourier light-field microscopy to separate signals with different periodicity in complex biological samples. This process allows for enhanced volumetric reconstruction and accurate image analysis.

1. Introduction

Our recently developed large-field Fourier light-field microscopy (FLFM) features rapid, high-resolution, volumetric in *vivo* imaging of live-cell or in vitro imaging since it enables retrieval of the entire sample volume form a single shot [1]. However, in some scenario, such as imaging beating heart in Xenopus, sparsity of FLFM image which benefits the 3D reconstruction can be impaired by the autofluorescence or fluorescence due to mislabeling from the stationary tissues around the moving target. To solve this problem, we here proposed a denoising-sparse decomposition method that can extract information of 3D morphological change from complex fluorescent signals and demonstrated the algorithm on imaging tadpole hearts with FLFM, separating the periodic moving cardiomyocytes from its surrounding stationary tissue, which enhances the imaging quality and allows precise measurement of ventricular volume at high temporal resolution. This algorithm promotes the power of FLFM on 3D in vivo imaging, aiding in cardiovascular study and pharmaceutical research using animal models.

2. Methods

2.1 System characterization

The imaging was conducted on our recently developed large-field FLFM system [2]. The homebuilt epifluorescence microscope was equipped with a Nikon water-dipping 16×, 0.80 NA objective lens and a light-emitting diode peaked at 470 nm. The native image plane was Fourier transformed using a Fourier lens and the back focal plane of the Fourier lens was partitioned by a 3 × 3 customized microlens array to form an array of elemental images on its back focal plane, which was recorded by a sCMOS camera (Fig. 1(a)).

2.2 Image processing

Raw images were initially denoised using a lab-written algorithm for pixel-wise noise correction in both spatial and time domain. Subsequently, the peak and trough of each cardiac cycle were determined based on image correlation. Further, stacks of every elemental image during one beating period were reshaped to a single 2D matrix M, with each of its columns being the light-field image at each frame. The matrix was decomposed to a low rank L, which represents the stationary part in the video, and a sparse component S, which represents the beating hearts, by solving an optimization problem using Robust principal component analysis [3]:

$$\min_{L,S} \|L\|_* + \lambda\|S\|_1, \text{ subject to } L + S = M$$

where $\|L\|_*$ is the nuclear norm of L, and $\|S\|_1$ is the L-1 norm of S. The sparse component was then reshaped back to the elemental image stacks and assembled to the light-field images for reconstruction.

2.3 Biological samples preparation

Xenopus laevis embryos were obtained by fertilizing wild-type oocytes with wild-type testis (ordered from Xenopus 1 Corp). The fertilized embryos at the 4-cell stage were micro-injected bilaterally with 0.5 ng H2b-GFP and 7.5 ng Ets1 MO in their marginal zone. On the day of experiment, embryos reached stage 46 were placed ventral side up on

an 3D printed bed in 0.1× MMR plus 0.1% MS222 and a 7 s (~14 cardia circles) video was taken at 100 Hz for each tadpole. All experimental procedures were performed according to 429 USDA Animal Welfare Act Regulations and were approved by Institutional Animal Care and Use Committee in compliance with Public Health Service Policy.

3. Results

By implementing this algorithm on the elemental light-field images (Fig. 1(b, c)), the moving cardiomyocytes were significantly separated from the stationary component, generating a sparser representation of the heart chambers (Fig. 1(d, e)). We then demonstrated a clear observation of the ventricle (the right chamber) and the truncus arteriosis (the left chamber) in the tadpole hearts (Fig. 1(f)) with volumetric reconstruction of the decomposed image, yielding a volume of approximately 600 μm × 600 μm × 400 μm. Noteworthy, the reconstruction following sparse decomposition (Fig. 1(h)) effectively eliminated background autofluorescence and mislabeled cells non-associated with cardiomyocytes compared to the reconstruction from undecomposed data (Fig. 1(g)). This allows for accurate segmentation of the cardiomyocytes and facilitates quantitative measurement of ventricular size, ejection fraction and cardiac output, key characteristics for evaluating heart development under gene regulation.

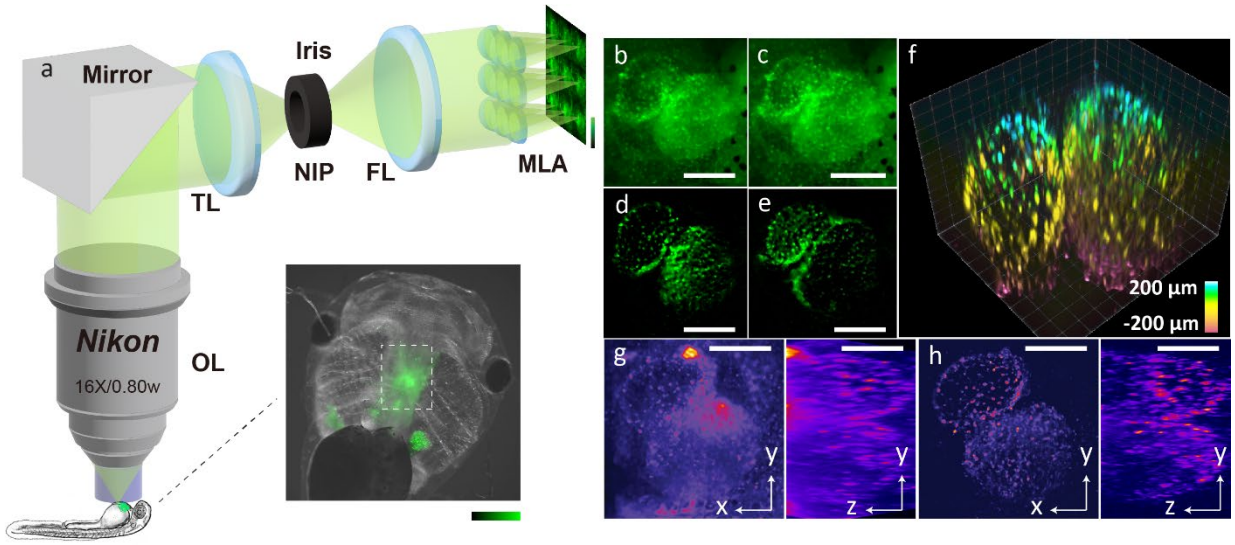

Fig. 1. (a) Experimental setup of the large field Fourier light-field microscopy system. OL, objective lens; TL, tube lens; FL, Fourier lens; MLA, micro lens array. (b), (c) Central elements of the raw light-field images at systole (b) and diastole (c) state. (d), (e) Central elements of the light-field images after decomposition on (b) and (c), respectively. (f) Color coded 3D image stack of the reconstructed volume. (g), (h) 3D max intensity projection (MIP) of reconstructed volume before (g) and after (h) decomposition. Scale bars: 200 μm (b-h).

4. Conclusion

In conclusion, we have developed a denoising-sparse decomposition framework for precise and ultrafast 3D in-vivo imaging of model animals using FLFM. This technology realizes image segmentation based on the targets' states of motion, increasing sparsity of FLFM images taken on live animals and yielding enhanced reconstruction outcomes. By applying this method to signals with varying periodicity, future investigations involving the labeling of multiple cell types can make use of both low-rank and multiple sparse components under a single illumination. This approach provides an additional channel for studying fast-paced events, such as cellular dynamics, morphological changes, and interactions between various biological entities.

5. References

[1] X. Hua, W. Liu, and S. Jia, "High-resolution Fourier light-field microscopy for volumetric multi-co lor live-cell imaging," Optica **8**, 614-620 (2021).
[2] W. Liu, G.-A. R. Kim, S. Takayama, and S. Jia, "Fourier light-field imaging of human organoids with a hybrid point-spr ead function," Biosensors and Bioelectronics **208**, 114201 (2022).
[3] E. J. Candès, X. Li, Y. Ma, and J. Wright, "Robust principal component analysis?," Journal of the ACM (JACM) **58**, 1-37 (2011).