Supplementary Code for

Fast, volumetric live-cell imaging using high-resolution light-field microscopy

Haoyu Li, Changliang Guo, Deborah Kim-Holzapfel, Weiyi Li, Yelena Altshuller, Bryce Schroeder, Wenhao Liu, Yizhi Meng, Jarrod B. French, Ken-Ichi Takemaru, Michael A. Frohman, and Shu Jia

User Manual for the Software

The software used for the light-field reconstruction in this work is based on the original software provided in Prevedel, *et al.* Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. *Nat. Methods* **11,** 727–30 (2014). In the publication, Prevedel and coauthors developed the software for light-field reconstruction using wave-optics based point-spread function deconvolution. The original software can be downloaded through:
https://www.nature.com/articles/nmeth.2964#supplementary-information

Here, we implemented key modifications for the use of HR-LFM based on the original software, stated as below:

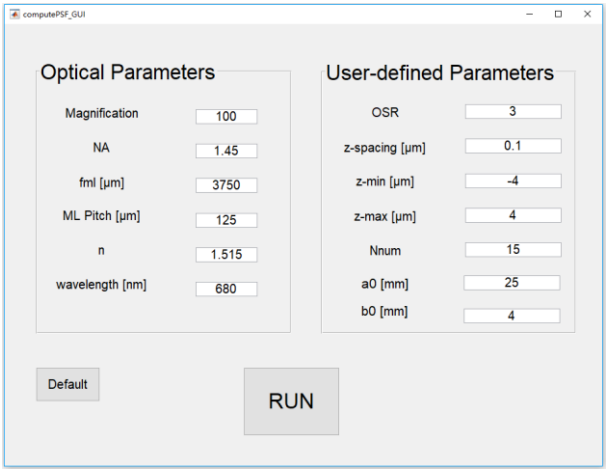
1. In HR-LFM, Fresnel propagation was considered for light-field propagation from the native image plane (NIP) to the microlens array (MLA) by *a*. After the phase modulation induced by the MLA, the second Fresnel propagation was considered to propagate the light field from the MLA to the camera by *b*. The codes that facilitate the new *a*/*b* optical design were added to the original version in:
   Lines 806-830 and 848-878, in *ComputePSF_GUI.m*,

2. There is an error in the original functions *calcPSF.m* and *calcPSFFT.m* describing the Debye integral equation. In these functions, the point-spread function (PSF) was generated *inversely* in the axial dimension in the object space. This worked for conventional LFM because of its axially-symmetric imaging scheme. However, this caused errors for HR-LFM due to its axially-asymmetric (or defocused) design. The two functions were corrected and replaced by *calcPSF_Corrected.m* and *calcPSFFT_Corrected.m*.

The users should replace these four files in the original software:
ComputePSF_GUI.m;
computePSF_GUI.fig;
calcPSF.m;
calcPSFFT.m
with the corresponding four files provided in this work, respectively:
ComputePSF_GUI.m;
computePSF_GUI.fig;
calcPSF_Corrected.m;
calcPSFFT_Corrected.m

Note 1: Input parameters for the user interface

In *PSF computation Module*, we added two parameters a_0 and b_0, representing the distances from the MLA to the NIP and the sCMOS camera, respectively. The initial values for HR-LFM were set as a_0 = 25 mm and b_0 = 4 mm. For conventional LFM, it should be set as a_0 = 0 mm and b_0 = 3.75 mm.



Note 2: Compatibility with MATLAB

The software is compatible with *MATLAB* 2014 and prior versions. For *MATLAB* 2017, the users should comment out or delete Lines 722-724 in *computePSF_GUI*, exemplified as:

```
721  %%%%%%%%% PREPARE PARALLAL COMPUTING %%%%%%%%%%%%%%%%%%%%%%%%%%%%
722  % if matlabpool('size') == 0 % checking to see if my pool is already open
723  %     matlabpool open
724  % end
725  %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

Note 3: Calculating the light field on the focal plane

The Debye theory was used for simulating the light-field output in a high-NA microscopy system. As described in Eq. (1) in **Methods**, Debye theory is given as:

$$U_i(\mathbf{x},\mathbf{p}) = \frac{\mathrm{M}}{f_{obj}^2\lambda^2}\exp\left[-\frac{iu}{4\sin^2(\alpha/2)}\right]\times\int_0^{\alpha}P(\theta)\exp\left[\frac{iu\sin^2(\theta/2)}{2\sin^2(\alpha/2)}\right]J_0\left[\frac{\sin(\theta)}{\sin(\alpha)}v\right]\sin(\theta)d\theta \quad (1)$$

where f_{obj} is the focal length of the objective, and J_0 is the zeroth order Bessel function of the first kind. The variables v and u represent normalized radial and axial coordinates; the two variables are defined by $v = k[(x_1-p_1)^2+(x_2-p_2)^2]^{1/2}\sin(\alpha)$ and $u = 4kp_3\sin^2(\alpha/2)$; $\mathbf{p} = (p_1, p_2, p_3)$ is the position for a point source in a volume in the object domain; $\mathbf{x} = (x_1, x_2) \in R^2$ represents the coordinates on the NIP; M is the magnification of the objective; the half-angle of the NA is $\alpha = \sin^{-1}(NA/n)$; and the wave number $k = 2\pi n/\lambda$ are calculated using the wavelength λ and the refractive index n of the immersion medium. For Abbe-sine corrected objectives, the apodization function of the microscope $P(\theta) = \cos(\theta)^{1/2}$ in this case.

Considering the light field at the focal plane of the objective (i.e. $p_3 = 0$), we obtained $u = 4kp_3\sin^2\left(\frac{\alpha}{2}\right) = 0$. Eq. (1) becomes:

$$U_i(\mathbf{x},\mathbf{p}) = \frac{\mathrm{M}}{f_{obj}^2\lambda^2} \times \int_0^{\alpha} P(\theta) J_0\left[\frac{\sin(\theta)}{\sin(\alpha)} v\right] \sin(\theta) d\theta \quad (2)$$

Eq. (2) indicates a real value for the wave function without complex phase information at the focal plane. Therefore, propagating the light field from the corresponding NIP to the MLA and camera will lead to incorrect results, thus an erroneous PSF calculation. Here, we recommended an alternative approach by using the PSF pattern at a plane slightly deviated from the focal plane (e.g. $p_3 = 50$ nm away from the focal plane in the object space) to replace the PSF pattern at the focal position (i.e. $p_3 = 0$).