Simultaneous whole-animal 3D-imaging of neuronal activity using light field microscopy

Robert Prevedel*, Young-Gyu Yoon*, Maximilian Hoffmann, Nikita Pak, Gordon Wetzstein, Saul Kato, Tina Schrödel, Ramesh Raskar, Manuel Zimmer, Edward S. Boyden and Alipasha Vaziri

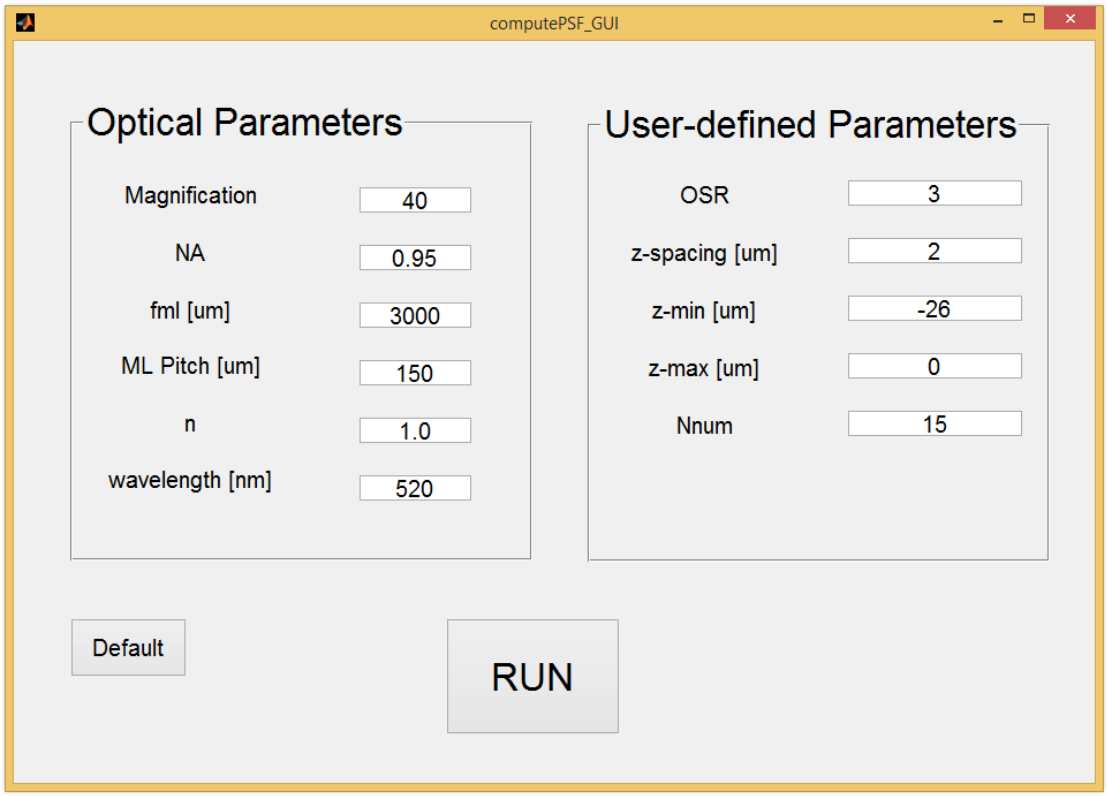
* These authors contributed equally to this work.
Correspondence should be addressed to E.S.B. (esb@media.mit.edu) or A.V. (vaziri@imp.ac.at).

User Manual for the Supplementary Software

This software package is a tool for 3D volume reconstruction from light field images. It was written in MATLAB and MATLAB 2014a or later is recommended to fully exploit parallel computing capability. The software capability was only verified for PCs with Windows 8.1 64-bit OS. For CPU computing, multi-core (ideally 16 or more) CPUs and at least 32GB of RAM are required. For GPU computing, one or more Nvidia CUDA-enabled GPUs with compute capability version 1.3 or higher is required. User might have to modify this software to run it on cloud/cluster computing environment.

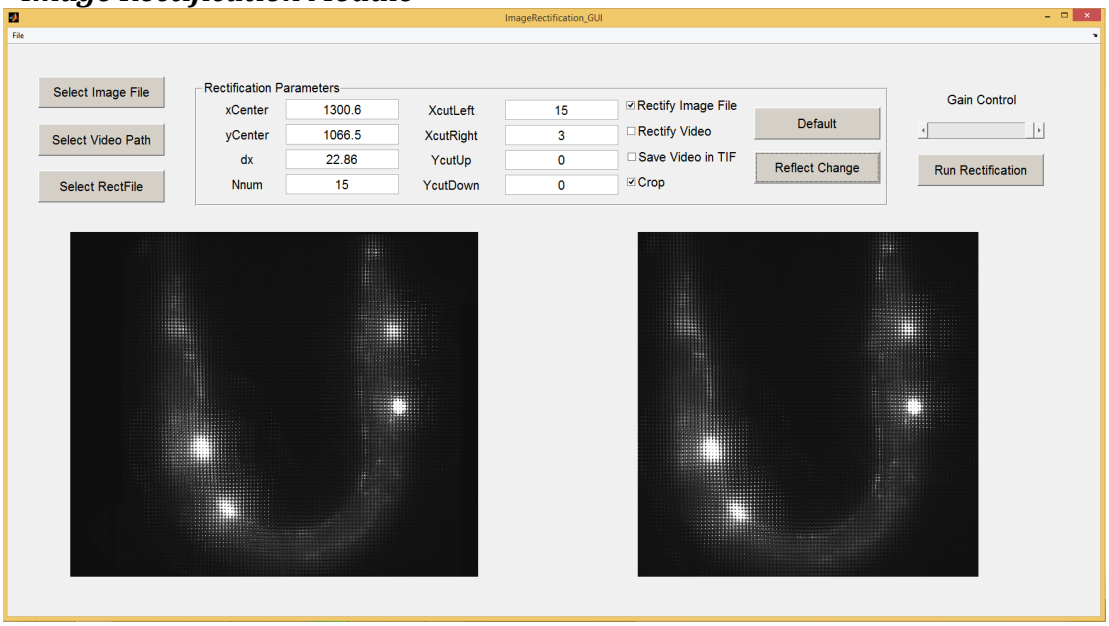
- **Input Data Structure**
  This software was not designed to be compatible with various data structures, so user might need to modify either the code or the input data to use it properly.
  - There should be 4 folders in the same path – *RUN, PSFmatrix, Data, Code*.
  - .mat files created by the software will be saved in *RUN* folder. In case the software doesn't seem to be working properly, it is recommended to try again after deleting the .mat files in this folder
  - PSF matrix files computed via PSF computation module will be saved in *PSFmatrix* folder. Only the PSF matrix files saved in this folder will be recognized by the reconstruction module.
  - Input data has to be saved in *./Data/01_Raw*/xxx/. Then, image rectification module will save the rectified result in *./Data/02_Rectified*/xxx/. User will be able to read the input from that folder using reconstruction module, and it will save the reconstructed result in *./Data/03_Reconstructed*/xxx/.
  - All input, either a single frame or a video, has to be saved in TIFF format. Each frame of the video has to be saved as a separated image file (The software will not be able to read from TIFF stack).
  - Video input (multiple TIFF image files) has to be in a same sub-folder (e.g., *./Data/01_Raw*/xxx/video/) and the name of each file needs to be as follows.
  DATANAMEXNNN.tif
  DATANAME: arbitrary string (should not include an alphabet X)
  X: indicator that the following number is the frame number
  NNN: frame number

- **Inputs to the software**
  The software has many input variables that need to be specified by the user. Many of them appear as abbreviated form without description, so users may refer to the following information to see what they mean.
  <PSF Computation Module>



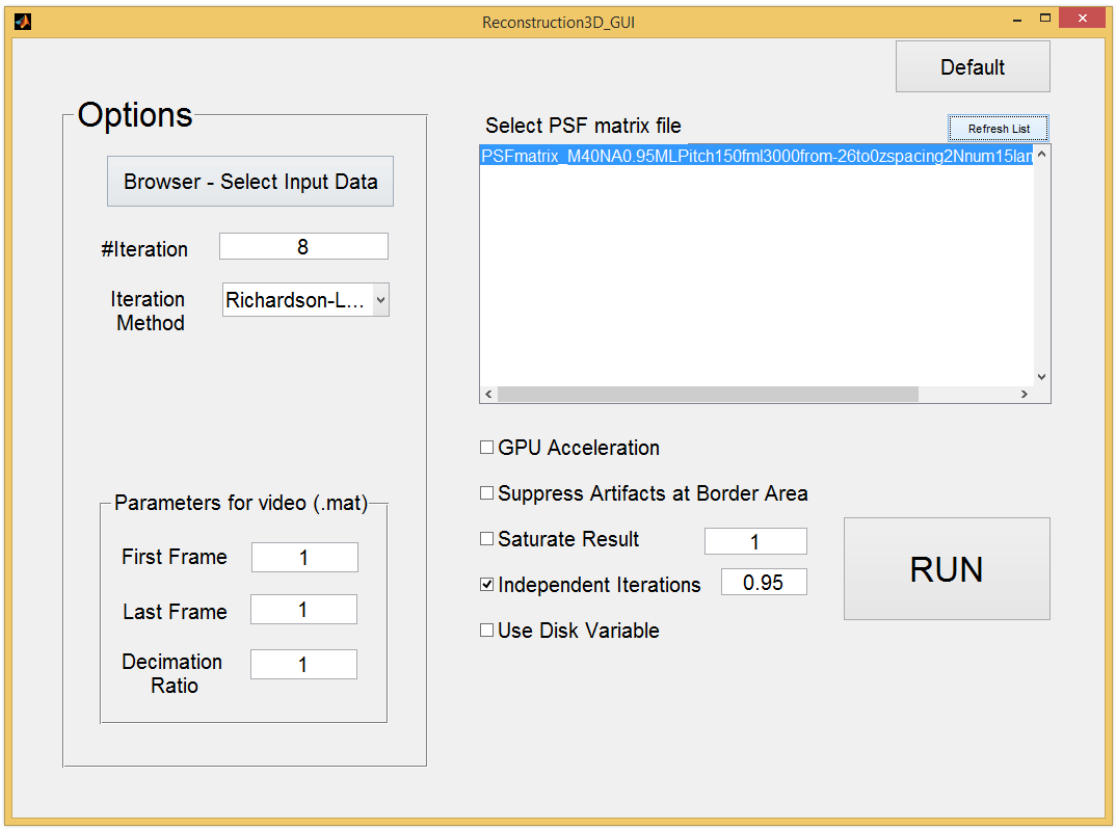
  - *Magnification* : magnification of the objective lens
  - *NA* : numerical aperture of the objective lens
  - *fml* : focal length of the microlens array
  - *ML Pitch* : pitch of the microlens array
  - *n* : refractive index of the immersion material
  - *wavelength* : wavelength of emission light
  - *OSR* : spatial oversampling ratio for computing PSF
  - *z-spacing* : spacing between adjacent z-planes
  - *z-min* : the axial location of the lowest z-plane with respect to the focal plane. Larger value indicates a plane that is farther from the objective lens where 0 corresponds to the exact focal plane.
  - *z-max* : the axial location of the highest z-plane with respect to the focal plane.
  - *Nnum* : number of virtual pixels (either in x or y direction) under each microlens (i.e., there will be Nnum x Nnum virtual pixels under each microlens). This has to be consistent with Nnum used for rectifying the input image

<Image Rectification Module>



- *Select Image File* : browse input image file to be rectified. This image will show up at bottom-left side of the panel. Input file has to be in TIFF format
- *Select Video Path* : browse a path that contains a series of input TIFF image files. More details about how the software recognize a video is explained in 'input data structure' part
- *Select RectFile* : User can either directly input the recficiation parameters (*xCenter, yCenter, dx*) or load a text file that contains these information. The text file can be generated using LFDisplay (http://graphics.stanford.edu/software/LFDisplay/).
- *xCenter* : x-coordinate of the center point of one of the "light field circles"
- *yCenter* : y-coordinate of the center point of one of the "light field circles"
- *dx* : distance between the "light field circles." This software assumes that the microlens array is perfectly aligned with respect to the pixel array (which is the image sensor) in terms of rotation.
- *Nnum* : number of virtual pixels (either in x or y direction) under each microlens (i.e., there will be Nnum x Nnum virtual pixels under each microlens). This has to be consistent with Nnum used for computing PSF
- *XcutLeft, XcutRight, YcutUp, YcutDown* : user can limit the field of view by adjusting these numbers. Each of these mean number of "light field circles" to be excluded at each side.
- *Rectify Image File* : Whether or not to save the rectified image file
- *Rectify Video* : Whether or not to rectify and save video input
- *Save Video in TIF*F : Whether or not to save rectified video in TIFF format. By default, rectified video will be save only in .mat format
- *Crop* : Whether or not to reflect field of view adjustment parameters (*XcutLeft, XcutRight, YcutUp, YcutDown*)
- *Gain Control* : the brightness of the image can be altered via this control bar. This change in brightness will not affect the rectification result

<Volume Reconstruction Module>



- *Browser – Select Input Data* : browse input data. The input has to be rectified properly using image rectification module
- *#iteration* : number of numerical iteration for volume reconstruction per each frame
- *Iteration Method* : Iterations method. Current version supports only Richardson-Lucy Iteration
- *First Frame, Last Frame, Decimation Ratio* : If the data is a time series in .mat format, user can decide the range for reconstruction as [FirstFrame:DecimationRatio:LastFrame]
- *Select PSF Matrix file* : Proper PSF matrix file has to be selected via this tab
- *GPU Acceleration* : Whether or not to compute on GPU
- *Suppress Artifacts at Border Area* : Whether or not to assign zeros to the border region which is often subject to artifacts
- *Saturate Result* : output will be automatically normalized to the full scale. User can decide to digitally "amplify" the results by activating this and assigning proper saturation gain
- *Independent Iterations* : Whether or not to run frame-by-frame independent reconstruction. If deactivated, the software will use the reconstruction result from last frame as the initial guess for the next frame. Before using the result from last frame, user can adjust its contrast to minimize artifacts. This option has to be used very carefully
- *Use Disk Variable* : Whether or not to save the result on the disk during reconstruction of a video. User might need to activate this option when processing large data set. SSD is strongly recommended