Instructions for the LabVIEW Simplex Algorithm Presented in:
Active PSF shaping and adaptive optics enable volumetric localization microscopy through brain sections

Main Code Name: Simplex-AO.vi

Other Files: The subvis required by Simplex-AO can be found in the folder "subvis". Additionally, there is an example file named Initial_Simplex_Guess_Example, containing initial, depth dependent guesses for the simplex algorithm.

Overview

The microscope used for this work had a piezo objective positioner, Deformable Mirror (DM) and sCMOS camera (see **Online Methods**) that sent and received information from this program. This program was originally part of a larger imaging program and the presented code was activated each time the simplex algorithm was called. A brief set of instructions and workflow covering the major sections of the code follows.

It is critical to note that the default settings provided here were highly specific to the equipment and set up used in the manuscript. Any differences in equipment, effective pixel size or imaging environment may dramatically alter the appropriate values.

Inputs

Automatically Read in by the Program:

When the vi is run, the program reads in the current focal position ("*Current Z*"), the coverslip position, a file for the Initial Simplex Settings (see **Notes**, below) and the current progress of the imaging sequence ("*Step #*" and "*Cycle #*"). With the default settings the depth is calculated as the difference between the coverslip and current focal position.

Required User Inputs:

The user needs to decide which optical aberrations to remove (see "*Aberration Selector*" on the front panel).

Optional User Inputs:

"*Use Adaptive Astigmatism*" increases the amount of astigmatism added to the DM when imaging deeper into the specimen. The values "*a*", "*b*", and "*c*" were determined from a fit of a 2nd order polynomial (astigmatism amplitude = $a*(depth)^2+b*depth+c$) to the data presented in Supplementary Figure 13.

The user can decide to restrict the ROI under optimization using the "*Use Cursor*" button and setting the "*boxsize*". The user can also decide to change thresholds or maximum iterations of the simplex algorithm.

The "*Use Simplex Results*" allows the user to employ previously determined AO corrections on the ROI. This option only works if the sample remains static and the field of view does not change.

Operation

Once the inputs are processed, the initial simplex estimates are read from the file, Initial Simplex Guess. As stated in the **Notes** below, this file contains a number of correction estimates based on the imaging depth to be used as initial guesses for the simplex algorithm. The depth value is the difference between the coverslip and current z position. These initial values will be displayed in the field "Simplex Amplitude" on the front panel.

Next, any added astigmatism (for obtaining 3D data), is removed prior to running the simplex algorithm.

Now, the Nelder-Mead Simplex Algorithm begins. A blinking, single molecule frame of data is recorded for each of the Initial Simplex Guesses and will be displayed on the front panel. The metric (see **Online Methods**) determines the quality of the PSF (variable named "*pval*", here). The "*pvals*" will be displayed in "*parray*" on the front panel.

The next step in the simplex algorithm is to perform a '*reflection*', in which a new simplex vertex is calculated based on the best (smallest "*pval*") initial simplex vertices. Another frame of raw data is recorded and the new metric is calculated.

The program shifts over to the 'cases' block in the Block Diagram, where the relationship between the recently calculated metric and "*parray*" will determine the next steps (see variable "condition") of the algorithm. Briefly, there are four possible paths; the '*reflection*' vertex is added to the "Simplex Amplitude" field or one of the '*expansion*', '*contract outside*' or '*contract inside*' fields are chosen in which a new vertex is calculated and evaluated with corresponding updates to the appropriate indicators on the Front Panel. Once this sequence is completed, the algorithm evaluates whether the "*pvals*" and "*Simplex Amplitudes*" have converged to beneath the thresholds.

If the values have not converged, the previous paragraph repeats until convergence is reached. Once the values have converged, the best performing (smallest "*pval*") simplex vertex is recorded. This vertex is displayed on the Front Panel (Final Simplex Values) and then applied to the DM. The astigmatism magnitude (for 3D imaging) that was removed in the second step is reapplied.

The above optimized simplex vertex is recorded in the DM settings along with the step number. For imaging thick sections requiring multiple simplex corrections, the previously determined values will be applied to the DM before the algorithm runs again.

Imaging after Simplex Optimization

The simplex DM settings are saved for the duration of the imaging sequence. For subsequent imaging cycles through the same ROI, the DM settings at different depths will be turned on or off based on the step number. Additionally, if the "Use Adaptive Astigmatism" was selected, the astigmatism magnitude will be adjusted depending on the depth of imaging.

Notes

Initial Simplex Guesses: There is an example text file included along with the software, named "Initial_Simplex_Guess_Example". The first row of this file contains depth values in microns. The remaining rows contain initial guesses for the simplex algorithm. The row

numbers correspond to the Zernike Mode (as defined by the Noll Indices). For example, oblique astigmatism has the Noll Index of 5 and will be in the fifth row below the depth values. There can be any number of columns (at least two are necessary). Ideally the first column will be at the minimum depth of 0 (coverslip) and a reasonable maximum imaging depth. There can be any number of columns provided the depth values monotonically increase.

Acknowledgements