Notes on convention and data format

General

1. Microscope:
    a. Widefield imaging: excitation wavelength of 488 nm; emission wavelength of ~532nm.
    b. Water-dipping objective: 1.2 NA, 60 x with a focal length of 3 mm.
    c. Magnification of the microscope is 83.3x, and the pixel size is 0.096 um (the physical size of each pixel on EMCCD is 8 um).

    There was a time we set the emission wavelength as 510 nm (or 550 nm), the objective NA as 0.8 NA and pixel size as 0.1625 um in the simulation – we don't use these configurations anymore.

2. In the phase reconstruction model, the indexing of Zernike coefficients follows Fringe convention , i.e., piston as 1st coefficient, tilts as 2nd and 3rd coefficients, etc. (https://docs.jcmwave.com/JCMsuite/html/ParameterReference/0c19949d2f03c5a96890075a6695b258.html?version=3.18.20).This is different from the microscope control software, where the piston is ignored and tilts are indexed as 1st and 2nd coefficients.

3. Standard Zernike bases (without normalization) are used in the calculation, where the effects of higher order coefficients are damped so the higher order aberrations are suppressed more if there is any penalty or regularization. We have verified that it works better than normalized bases. (We also preliminarily tried to use other weighting factors to damp the higher orders more or less, but have not found a better solution – maybe good to keep in mind to explore this a little more).

Simulated Data

A real microscopic image is taken as ground truth to generate simulated diversity images. Two simulated datasets (one with 2 diversity images and one with 3 diversity images) along with benchmarked results are provided for test purpose, the parameter settings can be found in the "InputParameters_2img.mat" or "InputParameters_3img.mat". To have a quick run the test data:

1) Configure the folder and output paths so the MATLAB can find the subfunctions and data.
2) Import "InputParameters_2img.mat" and run "test_simu_2img.m",
   or import "InputParameters_3img.mat" and run "test_simu_3img.m".

The scripts "test_simu_2img.m" and "test_simu_3img.m" are modified from the "test_recon_zern_simu.m" and are dedicatedly for the test data (so they are in the test data folder). Users can also configure in the scripts to add noise or modify the diversity phases.

Experimental Data

Two datasets were acquired from a layer of 1 um beads, with different input aberrations (unknow aberrations to be estimated). Currently, the phase reconstruction code (MATLAB based) and the microscope control software communicate via files only (images files and parameter files), so we modified the reconstruction code interface to read files as inputs. The information of the files is as following:

"beads_groundTruth.tif": the ground truth of the beads image (acquired without aberrations).

Folders **"beads_3"** and **"beads_25"**: the two datasets with different input aberrations.

And within each folder:

"beads_xx_IniAbr+offset.txt": initial setup of deformable mirror, not necessary for phase retrieval.

"beads_xx_coeff.txt": 5 rows of Zernike coefficients, including the input aberrations (row 1, to be estimated) and additional diversity phases (row 2 ~5).

beads_xx.tif: a stack of 14 images including raw images, diversity images and aberration corrected images as detailed in below table.

	Raw (Diversity 1)	Diversity 2	Diversity 3	Diversity 4	Diversity 5	Corrected	Raw (repeated)
Row in Coeff file	1	2	3	4	5	~	1
Number in image file	1,2	3,4	5,6	7,8	9,10	11,12	13,14

For each phase configuration, beads image was acquired twice -- the two images are supposed to be averaged to one to improve the robustness. The aberration corrected images were acquired after we applied the negative of the estimated aberration to deformable mirror to cancel the original input aberrations. In these cases, only two diversity phases/images (raw and diversity 2) were used to calculate aberration.

Subfolder "testResults_2img": the phase reconstruction results from two diversity phases/images (raw and diversity 2).

Please be aware:

1) The Zernike coefficients are indexed from tilts, i.e., tilts are as 1st and 2nd coefficients.
2) The coordinates system of the microscope and that of the reconstruction model (MATLAB code) are not matched. We need to perform a few operations on the experimental data (rotation and flipping on the images and convert certain Zernike coefficients to their negative values) to make the coordinates system consistent.

To address above issues, we have created new MATLAB scripts/functions to handle experimental data ("recon_zern_fileIO.m" and "test_recon_zern_fileIO"). The "recon_zern_fileIO.m" takes care of the

conversion of the coefficients indexing and the coordinates system, as well as other preprocessing procedures (background subtraction, cropping, edge smoothing).

To have a quick run on the test data, we have a simpler script “test_exp_2img.m” along within the data folder. Users only need to configure the paths and file names in the script. Note the provided test results were calculated from two diversity phases/images (raw and diversity 2), and only first 15 Zernike coefficients were estimated. The estimated Zernike coefficients were applied to the deformable mirror to cancel the original input aberrations and eventually to obtain the corrected images (#11,12 in the TIFF image file). While the results of “beads_25” dataset are less encouraging, the results of “beads_3” dataset are good.