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 ANSI convention, i.e., piston as 0^{th} coefficient, tilts as 1^{st} and 2^{nd} coefficients, etc. (https://en.wikipedia.org/wiki/Zernike_polynomials and https://www.telescope-optics.net/zernike_expansion_schemes.htm). This is different from the microscope control software, where Wyant convention is used and tilts are indexed as 1^{st} and 2^{nd} coefficients. Sometimes, both ANSI and Wyant conventions are supported, but the ANSI is the major one used through all codes.

    There was a time Fringe convention was used (before version 2), but now Fringe convention is no longer used.

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

Two ground truth images are provided for generating synthetic images: a resolution target image and a real microscopic image of a fixed cell with microtubule labeled. A simulated dataset based on the resolution target image (with 2 diversity image) along with benchmarked results are provided for test purpose, the parameter settings can be found in the "InputParameters.mat" and "test_recon_simu.m". To have a quick run on the test data:

1) Configure the folder and output paths so the MATLAB can find the subfunctions and data.
2) Import "InputParameters.mat" and run "test_recon_simu.m",

There are two scripts “test_recon_simu.m”: one in the main folder and one in the test data folder. They are almost same but the one in the test data folder is are dedicatedly for the test data.

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:

Folders **“beads_2”** and **“beads_8”**: 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_grdtrth_xx.tif”: the ground truth of the beads image (acquired without aberrations).

“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). Note these coefficients are configured for the deformable mirror, they might not be the exact ground truth aberrations in the actual images, but they are taken as “ground truth” just for reference.

beads_xx.tif: a stack of 5 images including raw images, diversity images as detailed in below table.

	Raw (Diversity 1)	Diversity 2	Diversity 3	Diversity 4	Diversity 5
Row in Coeff file	1	2	3	4	5
Number in image file	1	2	3	4	5

Subfolder “beads_mmImgCorr_xx”: the phase reconstruction results from **mm** diversity phases/images (raw and diversity 2).

“beads_mmImgCorr_xx.tif”: the aberration corrected image acquired by microscope (estimated the aberration from **mm** diversity phases/images, and then applied the negative of the estimated aberration to deformable mirror to cancel the original input aberrations).

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 of the wavefront and/or 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 ("test_recon_exp.m" and "recon_zern_fileIO.m"). 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_recon_exp.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 or three diversity phases/images, and only first 20 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. While the "beads_2" dataset uses 2 diversity images and get encouraging results, "beads_8" requires 3 diversity images to get good results.