**Phase Masking and Spatially-Variant Deconvolution Project**

Project hand-over notes compiled by Kaley McCluskey on 2017-03-31.

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**I. Tom Vettenburg’s SVD code**

* The up-to-date version (not counting any changes TV has made for M-Squared) is on the lab computer in Jonny’s GitHub folder.
* The main function is deconvolveCubicLightSheet.m. However, TV did not ‘package’ it nicely to index through folders and delete or re-process the .avi files, so I have done some simple packaging in another m-file:
* processVideos\_SpVarDeconv.m incorporates recursive indexing through folders, but none of the other functions in our normal processWaterImmersion… file. Most importantly, there is no ‘reprocessData’ option to turn off, so if you run processVideos\_SpVarDeconv on an avi file you have already processed, it will overwrite your .mat file.
* To simulate PSFs without analysing any data, I have created a pared-down version of the deconvolution code, CubicLS\_KMsimulation.m.
  + This is a flexible way to explore the effects of light sheet profile, phase mask strength, numerical aperture, etc., on system performance.
  + It could also be used to expand our exploration of the SVD technique itself to other, arbitrary phase masks, as well as the cubic ones we have currently defined.
  + The inputs to the code are:
    - experimentFilePath, the full path and file name of a real data file. The .avi will not be used, but the code needs access to the config information in the associated .json file.
    - LSalpha, the light sheet alpha value
    - PM alpha, the phase mask strength (more on that below)
    - excNA, the excitation NA, e.g. 0.42 or 0.6
    - detNA, the detection NA, which unlike excNA, is the detection iris diameter in meters, e.g., 0.003, 0.008. The iris diameter is converted to a numerical aperture in lines 46 and 80.
    - yRes, the number of subvolumes into which the imaging volume will be divided along the propagation axis. TV recommends always using an odd number so one subvolume will be centered. I typically trialed 5, 9, and 15.
    - yRes = 1 is the spatially invariant case. It takes up tons of memory, so if you are using it, reduce the viewSize to something less than the entire volume in line 59.
    - PMchoice, a dummy variable I added to toggle between the pupil functions of the two phase masks I was simulating. The pupil functions are defined in lines 90 and 92, and can be modified or replaced arbitrarily. At the moment the correspond to the two real phase masks we have for the system (see below).
  + In lines 230-240, you can select whether to save the output as a .mat file of the detection, light sheet, and restored PSFs, or simply to display it as a figure.
* I wrote a script to fit the PSFs and compare spot size and intensity across the field of view, but I recommend simply using Jonny’s version of the spot finder instead.

**II. The Experimental Phase Masks**

* We have two of them. They are kept in plastic boxes inside white cardboard LightForge containers, which are currently stored in the light sheet lab.
* The ‘2D’ phase mask has surface profile c\*(u^3 + y^3), strength 20.65, and active area 1 cm in diameter.
  + Note that Tom’s deconvolution code automatically takes into account the reduction in strength that comes from using less than the full active area, as long as the detection iris diameter is known (lines 89-90 of processVideos\_SpVarDeconv).
* The ‘General Cubic’ phase mask is the same size, and has surface profile c\*(u^3 + y^3)\* c \*(x^2\*y + x\*y^2) and strength 15.31.
  + c = lam\*alpha / (n-1) / r^3, where lam is the excitation wavelength, alpha is the phase mask strength, n is the refractive index, and r the detection aperture radius. The design values were lam = 532 nm, n = fused silica, and r = 0.005 m.
* When inserting a phase mask into the main LS system, I put a mirror in the sample chamber, focus a Gaussian beam onto it, and open the detection iris completely to ensure that I have a large spot to visually center in the active area of the mask.
* Any rotation of the mask needs to be recorded in the deconvolution code, but the sliding vertical posts of the holder should prevent small, accidental rotations. Just ensure that the mask is upright with the identifying text at the top and facing toward the objective, not the camera.
  + As of 2017-03-31, I have marked the top and bottom of an aligned phase mask with tape on one of the uprights. As long as only one of the uprights is loosened to remove/replace the mask, you should be able to return it to this exact spot, which will be useful when switching masks on a sample that you don’t want to move to align on a mirror.
* When simulating phase masks of varying strength, I generally used values between 3 and 14 for the General Cubic mask, and values between 6 and 28 for the 2D Cubic.

**III. Enumeration of Data Taken to Date**

* These folders will be stored on the lab computer on the H:\, F:\, or E:\ drives. I kept working notes on the experiment in a .txt file found inside each data folder.
* 2016-09-29\_cubicPM\_alphatest\_NAtest
* 2016-09-12\_DetectionIris
* 2017-03-15\_SVD
* 2017-03-16\_SVD
* 2017-03-30\_PhaseMasks \*The only one to actually use the phase masks. The rest are trialing different combinations of detNA/excNA/light sheet alpha.
* The data in 2017-03-30 has the unfortunate complication that detection NA and phase mask strength are coupled—in fact, changing the detNA is how changing PM strength is accomplished in this data set (by changing the illuminated footprint on the PM).
  + The way to decouple the two would be to replace one of the f = 50 mm lenses in the detection-arm collimator with a negative focal length lens, such that the beam was still collimated, but larger or smaller on the phase mask according to what lens was used.
  + To that end, we ordered plano-concave lenses with f = -30, -50, -75, and -100 mm, which are on the bench in the light sheet lab.
  + The footprint of the beam would still depend on the detection NA, but at least for a given detection NA, one could try 4-5 different phase mask strengths by changing the amount to which the collimator expands the beam.
  + This would need to be accounted for in the deconvolution code, which currently only calculates the effective PM strength based on its specified value and the detection iris diameter.