

Zernike Polynomial–Based Aberration Correction for Optical Vortex Beams

Overview

This guideline describes how to optimize and correct optical vortex beam quality in a two-photon (2P) holographic optogenetic setup using:

- Spatial light modulator (SLM) phase mask centering
- Zernike polynomial–based aberration correction
- Vortex quality (Q) evaluation
- Particle Swarm Optimization (PSO)

The goal is to produce bright, spatially uniform optical vortex patterns suitable for precise and safe photostimulation of neurons.

Preparation

Hardware Requirements:

- Custom 2P imaging and photostimulation setup
- Spatial Light Modulator (SLM)
- EMCCD camera
- Auto-fluorescent plastic slide (e.g., Chroma Technology)

Software Requirements:

- NeuroART software platform with aberration correction module (available online at: <https://github.com/losertlab/NeuroART>)
- MATLAB 2023 or later

Initial Setup:

- Mount the auto-fluorescent slide at the imaging plane. (e.g. use a red-fluorescent slide if photostimulation is done using opsins sensitive to red light)
- Ensure the SLM and EMCCD camera are correctly aligned and operational.
- Set the photostimulation laser to a low initial power level.

Step-by-Step Procedure

1. Initialize EMCCD acquisition

- Launch the EMCCD camera acquisition software (e.g., Micro-Manager).
- Lower the objective to focus on the auto-fluorescent slide.
- Set acquisition parameters: exposure time = 50 ms, gain = 10.

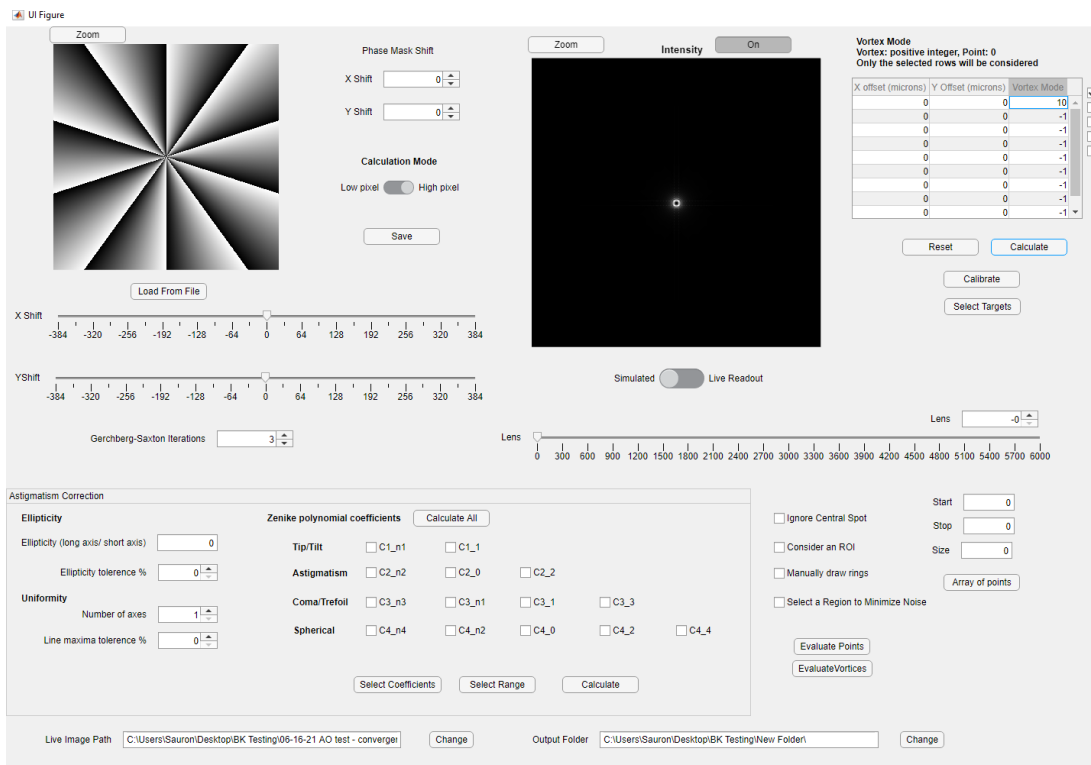
2. Configure the SLM parameters

- In NeuroART, open the folder BNS_SLM.

- Edit “dhot_opts.m” to match your photostimulation setup:
 - SLM dimensions and pixel count
 - Wavelength
 - Effective focal length

3. Launch the SLM control GUI

- Run “SLMcontrol_main.m” (in NeuroART/BNS_SLM) to open the SLM control interface.
- In the top-right table:
 - Set vortex beam size (L) = 10
 - Set lens value to 600 (adjust if your beam path’s focal length differs).



4. Generate a vortex beam

- Click calculate to project the corresponding vortex phase mask onto the SLM.
- Adjust the imaging objective’s z-focus until the EMCCD shows a sharply focused vortex pattern.
- Capture the fluorescence image of the generated intensity pattern through the EMCCD camera.

5. Visually evaluate vortex quality

- Check for a ring-like, uniform intensity distribution.
- If the pattern is uniform, no further corrections are needed.

- If the pattern appears distorted, wavefront aberrations are likely present. To compensate for these aberrations, run the following aberration correction routine before using neuronal samples for photostimulation.

6. Phase mask centering (first step of optimization)

- Run “centerPhaseMask.m”. This function uses Particle Swarm Optimization (PSO) to determine the optimal x and y coordinates for the SLM phase mask center. The quality of the generated vortex intensity pattern is evaluated at each iteration of the optimization process and serves as the objective function for the PSO algorithm. The quality metric (Q) is calculated by a separate MATLAB function, “vortexQuality.m”.
- After convergence, the script saves the optimal phase mask center coordinates to a CSV file (“phaseMaskCenter.csv”). These coordinates will be used in the subsequent steps of the aberration correction process.

7. Zernike polynomial–based aberration correction (second step of optimization)

- Run “zernike_aberrationCorrection.m” to compensate for higher-order aberrations. This step compensates for additional aberrations such as astigmatism, tip/tilt, coma, trefoil, and spherical aberrations.
- Since Zernike polynomials are orthogonal to each other, the optimization is performed one coefficient at a time. Because phase mask centering already corrects most of the distortions, this step explores only a narrow range of values (−0.02 to +0.02) for each Zernike coefficient.
- Once the optimization is complete, the function saves the set of Zernike coefficients that produces the most uniformly distributed vortex intensity pattern (saved in the csv file: “zernike_coeffs.csv”).
- These coefficients will be used in the SLM phase mask calculations during photostimulation experiments.