Extended-depth-of-focus for longer single-molecule tracking in cell nuclei

Type of position: Master's internship
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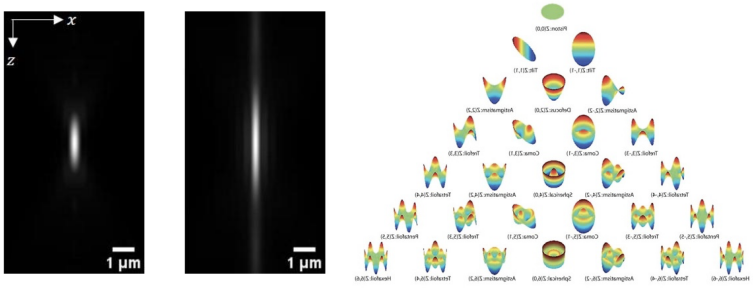
Internship Context and Objectives:

Single-molecule tracking (SMT) inside the **cell nucleus** is a powerful approach to probe **DNA repair mechanisms** and **transcription factor (TF) regulation** at the nanoscale. A central limitation in high-NA objectives necessary for single-molecule microscopy is the **short depth of focus (DoF)**, which causes tracked molecules to **leave focus prematurely**, shortening trajectories and biasing dynamic readouts.

This internship aims to **extend the depth of focus** in single-molecule localization microscopy (SMLM) and SMT **without sacrificing photons** by using a **deformable mirror (DM) at a pupil-conjugate plane** to implement **smooth, phase-only masks**. The goal is to achieve **2x-5x axial PSF extension** with **minimal lateral broadening** so that single emitters remain observable for longer, enabling **longer, less-biased SMT traces** in the nucleus.



Left, simulated point-spread function (PSF) with and without extended DoF. Right, Zernike polynomials that can be applied to a deformable mirror (DM).

Scientific approach:

- Engineer **smooth, phase-only masks** on a **pupil-conjugate deformable mirror** (log-asphere, spherical-blend, soft axicon).
- Target **2x-5x axial DoF** with **≤~1.5× lateral FWHM** and **~100% photon throughput**.
- Calibrate with **bead stacks** to build an empirical **PSF-vs-z model** for localization/tracking.
- Apply in **nuclear SMT** and quantify gains in **track length** and **z-robustness** over standard PSFs.

Intern tasks:

- **Design & simulation**: propose 2–3 DM mask candidates achieving 2×–5× DoF under lateral-blur constraints.
- **Implementation**: deploy masks on the microscope DM (pupil-plane alignment, closed-loop fitting).
- **Calibration**: acquire bead stacks; derive PSF model and CRLB/localization metrics.
- **Experiments**: perform nuclear SMT and matched reference recordings (astigmatic/standard).
- **Analysis and deliverables**: quantify trajectory gains (length, residence, diffusion states); release code + short report.

Candidate profile:

- Master 2 in physics/optics/biophysics/applied math or related field.
- **Required**: foundations in Fourier optics and microscopy; proficiency in Python or MATLAB for simulation/data analysis.
- **Desirable**: experience with SMLM/SMT, adaptive optics or DM control, wavefront sensing, single-molecule data processing, and basic cell handling.